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Off-Site Project?	No
Multi-center trial?	No
DSMB Involvement?	Yes
Tech Transfer:	CRADA

SYNOPSIS

Severe aplastic anemia (SAA) is a life-threatening bone marrow failure characterized by pancytopenia and a hypocellular marrow. Allogeneic bone marrow transplantation is curative in younger patients, but older age and/or lack of a suitable donor have limited application of this procedure. As an alternative to transplant, immunosuppressive treatment (IST) has provided durable remissions and similar long term survival [1]. Approximately 2/3 of patients who receive IST with horse anti-thymocyte globulin (h-ATG) and cyclosporine (CsA) have blood count recovery, but 25-30% do not respond and 30-40% will relapse. A likely explanation for partial recovery and relapse is incomplete elimination of auto-reactive T cells and insufficient stem cell reserve.

Thrombopoietin (TPO) is a key regulator of hematopoietic stem cell renewal and survival. To improve the hematologic response rate, our group assessed the addition of eltrombopag (EPAG), a synthetic mimetic of TPO, to IST in treatment naïve SAA. This combination achieved a higher complete response rate to about 50% and an overall response rate to 80%, both superior to historic controls [2]. This regimen received FDA approval in November 2018. Combined therapy is now being tested in a European randomized study. Furthermore, protocols have been developed internationally to determine whether EPAG and CsA, without ATG, are sufficient to improve blood counts, in countries where ATG is not available.

The long-term complications, relapse and clonal evolution, were no worse with the addition of EPAG than in our historical cohort, but still remain a problem. Clonal evolution occurs in 10-15% of patients and is defined as development of myelodysplastic syndrome or acute myeloid leukemia with characteristic cytogenetic abnormalities of aneuploidy, especially monosomy 7 or deletion 7q. There are no predictive tools to identify patients at higher risk for either of these two long term events.

Because SAA is a rare disease, treatment has been recommended to take place at a specialized center. However, delays in reaching such centers and initiating therapy are common. From current understanding of the disease, immune destruction of cells is ongoing during this period, likely impacting on both short and long term outcomes. We propose early initiation of lower dose CsA (2mg/kg/day) and EPAG to decrease ongoing immune destruction and stimulate HSPC while awaiting full work up and transfer to the Clinical Center (CC).

The aim of this study is to test feasibility and safety of initiating oral therapy before arriving to the NIH, based on diagnostic tests performed by local physicians and interpretation from experts here. Treatment will be initiated remotely but under complete guidance and supervision of the research team at the Hematology Branch. All patients except the ones who achieve complete response will receive standard three drug regimen upon completion of work up here at the CC. Primary endpoint of the study will be to assess feasibility and safety as a composite measure of misdiagnosis, non-compliance with the regimen or failure to establish care at the Clinical Center within 8 weeks of initiating treatment, and TRSAE (treatment related serious adverse events). Initial treatment period of 8 weeks may be extended in special circumstances (defined in section 9.6). Secondary endpoints are response rates at landmark time points, relapse, overall survival, and clonal evolution.

SCHEDULE OF EVENTS (SOE)

EVALUATION AND ASSESSMENTS	Screening /Baseline	Off Site Monitoring	1st Visit at Clinical Center	On Site Monitoring				Follow Up Visit									Relapse
PROTOCOL TIMEPOINT	1	2	3	5	6	7	8	11	12	13	14	15	16	17	18	19	20
Window +/- days	* +/- 4 weeks	+/- 8 Weeks	* +/- 8 Weeks					+/- 10D	+/- 10D	+/- 10D	+/- 10D	+/- 30D	+/- 90D	+/- 90D	+/- 90D	+/- 90D	
day /month				D 1	D 2	D 3	D 4	M1	M2	M3	M6	M12	M24	M36	M48	M60	
Past History & Consents																	
Review Inclusion/Exclusion Criteria	x																
Review Medical & Family History	x																
Physical Examination	x[3]									x	x	x	x	x	x	x	C
Review of Concurrent Medications	x[3]																
Vital Signs				x	x	x	x										
PPD			x[2]														
NHLBI Screening Consent	x																
Consent to study	x																
CHEMISTRY LABS																	

EVALUATION AND ASSESSMENTS	Screening /Baseline	Off Site Monitoring	1st Visit at Clinical Center	On Site Monitoring				Follow Up Visit									Relapse
PROTOCOL TIMEPOINT	1	2	3	5	6	7	8	11	12	13	14	15	16	17	18	19	20
Window +/- days	* +/- 4 weeks	+/- 8 Weeks	* +/- 8 Weeks					+/- 10D	+/- 10D	+/- 10D	+/- 10D	+/- 30D	+/- 90D	+/- 90D	+/- 90D	+/- 90D	
day /month				D 1	D 2	D 3	D 4	M1	M2	M3	M6	M12	M24	M36	M48	M60	
Female, Pregnancy Test (blood or urine)	x[1]		x							x	x	x	x	x	x	x	x[3]/C
Acute care/mineral panels	x[3]	x[3]	x		x		x			x	x	x	x	x	x	x	x[3]
Hepatic Panel	x[3]	x[3]	x		x		x			x	x	x	x	x	x	x	x[3]
LDH panel	x[3]		x		x		x			x	x	x	x	x	x	x	x[3]
Total Protein, uric acid, CK			x														
Thyroid Function Test			x														
Folate Level, B12 Level			x														
Iron Panel (ferritin, transferrin, % saturation)			x							x	x	x	x	x	x	x	
HEMATOLOGY LABS																	
CBC with differential	x[3]	x[3]	x	x	x	x	x	x[3]	x[3]	x	x	x	x	x	x	x	x[3]
Reticulocyte Count	x[3]	x[3]	x					x[3]	x[3]	x	x	x	x	x	x	x	x[3]

EVALUATION AND ASSESSMENTS	Screening /Baseline	Off Site Monitoring	1st Visit at Clinical Center	On Site Monitoring				Follow Up Visit									Relapse
PROTOCOL TIMEPOINT	1	2	3	5	6	7	8	11	12	13	14	15	16	17	18	19	20
Window +/- days	* +/- 4 weeks	+/- 8 Weeks	* +/- 8 Weeks					+/- 10D	+/- 10D	+/- 10D	+/- 10D	+/- 30D	+/- 90D	+/- 90D	+/- 90D	+/- 90D	
day /month				D 1	D 2	D 3	D 4	M1	M2	M3	M6	M12	M24	M36	M48	M60	
Direct Antiglobulin Screen			x														
Type and Screen	x[3]		x														
Viral Serologies																	
Hepatitis A, B, C			x														
HIV			x														
HSV			x														
EBV and CMV			x														
Other Blood & Bone Marrow Tests																	
Lymphocyte phenotyping T, B, NK			x							x	x	x	x	x	x	x	x[3]
Telomere length of leukocytes			x														
Flow Cytometry of peripheral blood			x							x	x	x	x	x	x	x	x[3]
HLA typing			x														
Cyclosporine Level			x														x[3]

EVALUATION AND ASSESSMENTS	Screening /Baseline	Off Site Monitoring	1st Visit at Clinical Center	On Site Monitoring				Follow Up Visit									Relapse
PROTOCOL TIMEPOINT	1	2	3	5	6	7	8	11	12	13	14	15	16	17	18	19	20
Window +/- days	* +/- 4 weeks	+/- 8 Weeks	* +/- 8 Weeks					+/- 10D	+/- 10D	+/- 10D	+/- 10D	+/- 30D	+/- 90D	+/- 90D	+/- 90D	+/- 90D	
day /month				D 1	D 2	D 3	D 4	M1	M2	M3	M6	M12	M24	M36	M48	M60	
Bone Marrow aspiration and core biopsy for standard morphologic analysis	x[8]		x							x	x	x	x	C	C	C	x[3]/C
Bone Marrow for chromosomal analysis			x							x*	x*	x	x	C	C	C	X[3]/C
Telephone Weekly Follow Up (+/- 3 days) Appendix B																	
Clinical Assessment A-F with progress notes		x															
Laboratory Assessment A-D		x															
Review of Medications		x															
Local Physician Involvement		x															
STUDY MEDICATION																	
Cyclosporine	x[5]			x[6]							x[7]		x[8]				x[5]

EVALUATION AND ASSESSMENTS	Screening /Baseline	Off Site Monitoring	1st Visit at Clinical Center	On Site Monitoring				Follow Up Visit									Relapse
PROTOCOL TIMEPOINT	1	2	3	5	6	7	8	11	12	13	14	15	16	17	18	19	20
Window +/- days	* +/- 4 weeks	+/- 8 Weeks	* +/- 8 Weeks					+/- 10D	+/- 10D	+/- 10D	+/- 10D	+/- 30D	+/- 90D	+/- 90D	+/- 90D	+/- 90D	
day /month				D 1	D 2	D 3	D 4	M1	M2	M3	M6	M12	M24	M36	M48	M60	
Eltrombopag	x												x[8]				x
h-ATG				x	x	x	x										
EVALUATIONS																	
EKG (prior to h-ATG)			x														
Central Line Placement				C													
Chest X-Ray			C														
Research Samples																	
60 mL blood sample (3 ml/kg not to exceed 60 ml for pediatric)			x							x	x	x	x	x	x	x	x
15 mL bone marrow aspirate sample			x							x	x	x	x	x	x	x	
10 mL additional blood sample (3 ml/kg not to exceed 60 ml for pediatric)										x	x	x	x	x	x	x	

EVALUATION AND ASSESSMENTS	Screening /Baseline	Off Site Monitoring	1st Visit at Clinical Center	On Site Monitoring				Follow Up Visit									Relapse
PROTOCOL TIMEPOINT	**1**	**2**	**3**	**5**	**6**	**7**	**8**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Window +/- days	*** +/- 4 weeks**	**+/- 8 Weeks**	*** +/- 8 Weeks**					**+/- 10D**	**+/- 10D**	**+/- 10D**	**+/- 10D**	**+/- 30D**	**+/- 90D**	**+/- 90D**	**+/- 90D**	**+/- 90D**	
day /month				**D 1**	**D 2**	**D 3**	**D 4**	**M1**	**M2**	**M3**	**M6**	**M12**	**M24**	**M36**	**M48**	**M60**	

*** = if patients are available to come within 72 hours of referral then baseline and 1st visit at Clinical Center will be performed at same time.**

*** = Grace period allows extension of this time frame to report to the Clinical center in special circumstances**

x^1 = women of childbearing potential and within 2 weeks of initiation h-ATG

x^2 = per PI discretion: at risk subjects only, based on history or geography and maybe performed off site

x^3 = outside labs results accepted

x^4 = not required if clinically indicated

x^5 = dose is 2mg/kg/day

x^6 = dose increase based on age, section 5.1

x^7 = responders will have dose decrease, 2 mg/kg/day

x^8 = drug discontinued

X^9=outside bone marrow results accepted after verification at the NIH

x*= Bone marrow aspiration, biopsy, and chromosomal analysis may be omitted if one was performed within 4 weeks of the timepoint analysis.

C = Considered optional

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any modification to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 OBJECTIVE

Primary Objective: To determine feasibility and safety of early initiation of oral therapy, cyclosporine (CsA) and eltrombopag (EPAG), in treatment naïve patients with severe aplastic anemia (SAA).

Secondary Objective: To assess response, relapse and clonal evolution.

2 BACKGROUND AND SCIENTIFIC JUSTIFICATION

2.1 PATHOPHYSIOLOGY OF APLASTIC ANEMIA

SAA is a serious hematologic disease characterized by pancytopenia and hypocellular bone marrow. It has been associated with certain chemicals, pregnancy, and seronegative hepatitis but there is no etiology identified in most cases [1] The bone marrow almost completely lacks hematopoietic and precursor stem cells (HPSC), detected by CD34 antigen. Years of clinical and laboratory research have identified immune destruction of HPSC to be the main mechanism of disease[3] Aberrant increase in activated cytotoxic T-cells particularly oligoclonal expansion of high antigen-affinity effector memory T cells (CD8+CD28-CD57+) are found by flow cytometry and deep sequencing [4, 5]. These cytotoxic T cells in turn exert their effect by type 1 cytokine production, Interferon gamma (IFN γ), via Fas ligand mediated apoptosis [6, 7]. On the other hand, the regulatory T cells (Tregs) are low in AA patients on presentation and increase with hematologic response to immunosuppressive therapy. [8, 9] [10].

2.2 CLINICAL CONSEQUENCES OF APLASTIC ANEMIA

Clinical symptoms correlate with severity of cytopenia. Neutropenia, when severe, results in susceptibility to bacterial and fungal infections, which account for early morbidity and mortality. Thrombocytopenia presents as mucocutaneous bleeding, including epistaxis, gum bleeding, and petechiae/ecchymosis; but rarely as life threatening gastrointestinal and intracranial hemorrhage. Anemia leads to inability to perform daily activities over time. Patients often have chronic transfusion requirements for both red blood cells and platelets, exposing them to risks of transfusion overload, acute and delayed transfusion reactions, and alloimmunization.

2.3 TREATMENT OF APLASTIC ANEMIA

2.3.1 Allogeneic Hematopoietic Stem Cell Transplantation

Allogeneic hematopoietic stem cell transplantation provides cure by rapidly restoring hematopoietic and immune systems in majority of the patients. HLA-matched related donor (MRD) transplant provide long term survival reaching 80% in younger patients, <40 years of age [11]. The occurrence of graft-versus-host disease is age dependent and continues to be the major limiting factor in terms of morbidity and mortality as well as long-term quality of life [12].

Historically, matched unrelated donor (MUD) transplantation has not been recommended due to high rates of GVHD and delay in identifying and recruiting a donor [11]. Recent data suggests improved 2 year overall survival and event free survival with MUD as first line treatment compared with IST or MUD after IST failure in younger patients [13]. Peripheral bone marrow source, older age and the interval from diagnosis to treatment beyond 6 months were strongest predictors of poor outcomes [11, 14]. For adults, IST is the standard of care in absence of MRD transplant options. Conversely, children have excellent survival with unrelated grafts [15].

Therefore, MUD transplant is recommended as first line treatment by some experts in pediatric patients if no delay is anticipated [1].

HLA- haploidentical transplant is an attractive option for many patients. The use of posttransplant cyclophosphamide (PT-CY) conditioning has produced promising outcomes in small studies [16] [17]. Currently, it remains investigational and is only recommended after IST failure and if an HLA-matched donor is unavailable [1]. Studies combining haplo-identical donor and umbilical cord transplant are underway at NIH and other institutions.

2.3.2 Immunosuppression with horse Anti-thymocyte Globulin (ATGAM®; h-ATG) and Cyclosporine (CsA)

With the recent FDA approval, EPAG + IST is now the standard treatment for newly diagnosed SAA. IST with CsA and ATG results in hematologic recovery in 60-70% patients leading to a 5 year survival of 80-90% among responders [3]. The mechanism by which h-ATG improves bone marrow failure in aplastic anemia is not fully understood. ATG preparations contain a variety of antibodies to human T cell epitopes, many directed against activated T-cells or activation antigens [18] [19]. Decrease in circulating lymphocytes after ATG is transient but number of activated T-cells are decreased for prolonged periods of time; this effect is also reflected in decreased lFN-γ and tumor necrosis factor (TNF) [20]. Although ATGs are mild lymphodepleting agents, even minor differences have clinical impact as highlighted by lower response rates with rabbit ATG compared to horse ATG in treatment naïve patients with SAA [21]. At our institution, a dose of 40mg/kg/day for 4 days has been used since 1995 with consistent and similar response rates to other groups, who utilize different dosage, formulation and duration of treatment [2, 22].

In contrast to ATG, CsA has a selective inhibitory effect on T lymphocytes, suppressing early cellular response to antigenic and regulatory stimuli. By blocking expression of nuclear regulatory proteins, it leads to reduced T cell proliferation and activation with diminished release of cytokines such as interleukin-2 (IL-2) and IFN-γ [23, 24] Although CsA and h-ATG combination is superior than either agent alone, cyclosporine monotherapy is commonly utilized worldwide due to lack of ATG preparations, less toxicity, oral route of administration, and relatively inexpensive cost. Previous large randomized trials comparing CsA to ATG or CsA and ATG demonstrated an ORR of 40-45% in CsA alone arm at 6 months [25] [26] [27]. These results have been replicated by many recent studies around the world. [28] [29] [30]. In a small study of 6 patients from Netherlands, cyclosporine monotherapy was shown to yield hematologic response even in patients with very severe aplastic anemia [31]. Many attempts to improve the response rates achieved by this combination had failed until the addition of EPAG.

2.3.3 Eltrombopag

Eltrombopag (SB-497115-GR, Promacta®), the bis-monoethanolamine salt form, is an orally bioavailable, small molecule 2nd generation thrombopoietin receptor (TPO-R) agonist. EPAG has been shown to increase platelets in healthy subjects, patients with chronic immune thrombocytopenic purpura (ITP), with hepatitis C associated thrombocytopenia [32] and in refractory SAA as single agent and in treatment-naïve SAA in combination with IST [33] [2] Thrombopoetin (TPO), a glycoprotein class 1 hematopoietic cytokine, is the main regulator of HPSC self-renewal and survival. Upon binding to its receptor, c-mpl, TPO activates cell signaling through JAK-STAT and other pathways. This process is tightly balanced by negative regulatory signaling. Recent studies indicate that chronic exposure of HPSCs to IFN-γ, as exemplified in

subjects with SAA, impairs self-renewal by perturbing TPO signaling pathways. Despite elevated levels of TPO in subjects with SAA, EPAG improves trilineage hematopoiesis by binding to a non-competitive receptor [34] EPAG, unlike TPO, evades HPSC inhibition caused by chronic inflammatory conditions (Alvarado L. Blood. 2017 – Abstract). Recent data also demonstrates that intracellular iron chelation property of EPAG contributes to its stem cell stimulatory effect [35]

Based on our work here at NIH, EPAG is FDA approved for SAA in first line with IST (2018) and in refractory setting (2014). In a phase I/II dose escalation trial, nearly half of the patients with refractory SAA demonstrated hematologic response in at least one lineage at 3 months [36]. Long term analysis of the extension phase including 43 patients showed an ORR of 40% at 3 months [33], and an additional 26% by 6 months (Abstract, Winkler T Blood. 2017). In treatment naïve SAA patients, EPAG added to immunosuppression resulted in complete hematologic response that was significantly better than historical rate of 10%. Long term outcomes, relapse and clonal evolution, were 14% and 8% respectively at 2 years [2]. Common side effects included liver function abnormalities, and diffuse erythematous skin rash. There were no additional toxicities noted with the combination.

Combination of CsA and EPAG are often used for relapse treatment in 12-H-0150 protocol with good hematologic recovery in majority of the patients. There were no unexpected adverse events, in fact, LFT abnormalities were observed less frequently (unpublished data).

2.3.4 Rationale for Dose Selection

Eltrombopag 150mg orally once daily is selected as the starting dose based on safety and efficacy data from two prior studies conducted here at NIH. In a phase II (NCT00922883) trial with refractory SAA patients, EPAG proved to be efficacious without dose limiting toxicities at a dose of 150mg. Furthermore, no increased or unexpected toxicities were noted with Eltrombopag, CsA, and h-ATG combination (12-H-0150). Pharmacokinetics data indicate higher plasma drug concentrations with regular dose of EPAG in patients with East Asian and Southeast Asian ancestry, therefore, a starting dose of 75mg daily will be administered as in our previous protocols.

In our previous protocols, a starting dose of cyclosporine 3mg/kg twice a day is given (higher dose) with a target drug level between 200 and 400mcg/L. For this protocol, we plan to administer 2mg/kg/day (lower dose). CsA has been shown to work in SAA at varying doses, schedule, and therapeutic trough. However, dedicated dose escalation studies to guide CsA dosing are lacking. At the clinical center, initial starting dose of 6mg/kg/day has been prescribed historically whereas doses as high as 12mg/kg/day have been used in German trials [37]. Therapeutic goals have also varied among trials from different institutions, and in developing countries the dose is typically adjusted based on toxicities. Lower dose CsA is not studied previously as the starting dose but we have observed ongoing hematologic response and delay in relapses with prolonged administration of lower dose CsA after initial response [38]. Lower dose CsA resulted in suppression of cytotoxic T cells and increase in T regs in patients with atopic dermatitis, another chronic autoimmune condition, suggesting a degree of immunosuppression with this dose [39].

Lower dose CsA is also safer to administer because of less toxicities and no requirements to follow levels, which may not be readily available in certain outpatient settings. Patients will only continue 2mg/kg until the initiation of standard therapy at CC, at which time the dose will be increased to standard 3mg/kg twice a day with a target goal between 200 and 400mcg/L to complete 6 months.

After 6 months, CsA will be decreased to 2mg/kg for 18 months to complete 2 years as employed in 12-H-0150.

2.3.5 Scientific and Clinical Justification of the Protocol

The current protocol aims to test the risks and benefits of early treatment with orally administered drugs in patients who have delay in receiving standard treatment, a combination of CsA, EPAG and h-ATG. The desirability of more expeditious treatment is based on the pathophysiology of immune aplastic anemia, T cell mediated destruction of hematopoietic stem and progenitor cells leading to a deficit in marrow regenerative function. Both drugs individually have activity in aplastic anemia as single agents [33, 36, 40] and carry low risk safety profiles over the short term. Indeed, the combination of eltrombopag with cyclosporine, without ATG, is currently being tested in a multicenter trial (SOAR trial: NCT02998645).

Appropriate treatment of patients with SAA is often delayed, as most physicians and many hematologists are not familiar with the disease or comfortable with its therapy. As a quaternary referral center for marrow failure, entry of patients to our protocols maybe delayed for logistical reasons. Even when patients can be admitted to the Clinical Center relatively proximate to their diagnosis, institution of effective therapy is often postponed while awaiting results of testing, intravenous catheter placement, or evaluation of complications or comorbidities. During this period, absent definitive therapies, stem and progenitor cell destruction is assumed to be ongoing.

Morbidity and mortality in severe aplastic anemia patient results from severity and duration of pancytopenia. Expeditious commencement of therapy is expected to abbreviate the period of severe pancytopenia with its attendant consequences: infections and treatment of febrile neutropenia, bleeding from ongoing thrombocytopenia, and anemia. Furthermore, rapid institution of treatment should also allow patients who do not respond to medical therapy, or whose response is not optimal, to proceed faster to transplantation options. Shorter periods between diagnosis and bone marrow transplantation have repeatedly been shown to correlate with transplant outcomes [41]. Robust and rapid hematologic recovery has been associated with better survival outcomes [42], and in our experience, complete hematologic responders are less likely to evolve to myelodysplasia or leukemia (unpublished data).

In the current protocol, CsA and EPAG will be initiated after consent is obtained by NIH physicians by audio/video communication (telephone calls, skype etc.) or in person at the Clinical Center if able to arrive here within 72 hours for initial appointment. Hematology branch team will follow the patients actively with every week telephone follow ups until patient receives standard treatment at the NIH. Drugs will be provided by the Clinical Center; both physicians and patients will be informed about side effects, monitoring requirements (clinical and laboratory), and measures that may lead to discontinuation of treatment.

Urgent treatment in absence of full information or the availability of all effective drugs, has precedents in medicine and within hematology, based on the benefits of immediate therapy and the low risk of the drugs employed. Most notably, acute promyelocytic leukemia, a highly fatal and aggressive form of acute myeloid leukemia, has been successfully treated achieving complete response rates up to 90-95% and disease free survival up to 86% with early recognition and rapid initiation of all-trans retinoic acid (ATRA) based treatments [43]. Other fatal diseases with rapid treatment while awaiting complete testing include heparin induced thrombocytopenia, and catastrophic anti-phospholipid syndrome. Employing similar concept, we believe rapid treatment

of SAA will decrease short term complications of pancytopenia, and long-term complications of clonal evolution and relapse rates by salvaging HSPCs and shortening the duration of chronic immune destruction.

The combination of ATG, which lyses lymphocytes, CsA, which blocks T-cell function, and EPAG, which stimulates stem cells, has produced excellent survival rates, comparable to bone marrow transplantation. In our experience, there are beneficial short-term effects of this combination: blood counts improve rapidly, decreasing the transfusion requirement and risk of neutropenic fever and infections. For EPAG added to IST in ongoing Hematology Branch trial, the cumulative relapse rate is ~40% and the rate of clonal evolution (progression to MDS/AML, or cytogenetic abnormalities) is approximately 15%, both comparable to IST alone. EPAG added to immunosuppression has significantly increased complete response rates, however, relapses and clonal evolution still remain a problem. As noted above, this may be mitigated by early initiation of immunosuppression and EPAG to stabilize immune destruction and stimulate hematopoietic stem cells.

For purposes of analysis, the major endpoints of the study will be the feasibility and safety of the design for expeditious treatment. This will be assessed as a composite measure of misdiagnosis, TRSAE, and non-compliance with the regimen or failure to establish care at the CC within 8 weeks of initiating treatment. Overall and complete response rates will be analyzed to be comparable to those established in standard protocols at the NIH for SAA.

2.3.7 Justification for Extension Cohort

Protocol specified interim analysis and recent re-analysis after accrual of 36 subjects were performed. Primary endpoint to assess the safety and feasibility of early oral therapy initiation was met with 0 composite events (non-compliance, treatment related serious adverse events, or misdiagnosis). Secondary endpoints including efficacy, relapse and clonal evolution rates were also re-analyzed after 36 patients. Compared to our previous protocol (12-H-0150 – cohort 3 and extension; Standard group) in which ATG, EPAG and CSA were all started on Day 1, baseline characteristics were similar in the Early group (20-H-0033) in terms of age, gender, and ethnicity however more patients with very severe aplastic anemia were enrolled in Standard group (52%) versus Early group (28%; Table 1). At 6 months, ORR (80% v 83%), CR (40% v 50%) and PR (33% v 40%) were equivalent in Standard and Early group, respectively (Figure 1). Median time on oral therapy was 14 days (range 0,86) and even with this short time on oral therapy, we observed a significant increase in absolute neutrophil counts, a trend towards increased absolute reticulocyte counts and decreased lymphocyte counts between pre-treatment and end of oral therapy (before hATG).

While relapse rates were similar between the two groups, high-risk clonal evolution, defined as acquisition of chromosome 7 abnormalities or morphologic diagnosis of myeloid neoplasm, was observed at much lower rate at median follow-up time of 363 days in Early group compared to Standard group (3.8% vs 8%). In EPAG treated patients in Standard group, most high-risk evolution occurred within the first 6 months so the 50% lower rate observed in Early group is striking. High-risk clonal evolution is a major contributor of mortality (Groarke EM. Leukemia. 2023) in SAA patients treated with IST. The lower rates observed in this study maybe by chance due to small sample size or biologically explained by reduction in regenerative stress by halting immune attack earlier in the disease and preserving stem cell pool or due to immunomodulatory activity of CSA and/or EPAG prior to hATG.

In the extension cohort, we continue with the same study design and eligibility criteria but plan on accruing an additional 41 subjects to specifically address the long-term complications, relapse and high risk clonal evolution.

Table 1: Patient characteristics	Early initiation (n=36)	Standard therapy (n= 139)
Age (median, range)	37 (8, 69)	33 (3, 82)
<18 years (n, %)	5 (14)	25 (18)
18-40 years (n, %)	19 (53)	51 (37)
>40 years (n, %)	12 (33)	63 (45)
Gender		
Male (n, %)	20 (56)	66 (47)
Female (n,%)	16 (44)	73 (53)
Race (n, %)		
White	16 (44)	77 (55)
Black or African American	16 (44)	34 (24)
Asian	2 (6)	10 (7)
Multiple Races	1 (3)	7 (5)
Unknown	1 (3)	5 (4)
Other		6 (4)
Severity of disease		
SAA (n, %)	**26 (72)**	**67 (48)**
VSAA (n, %)	**10 (28)**	**72 (52)**
Presence of PNH clone (GPI negative neutrophils >1%, n, %)	16 (44)	62 (45)
Pre-treatment clinical blood counts		
Absolute neutrophil counts (median, range), K/uL	0.3 (0, 3.6)	0.2 (0, 20)
Platelet counts (median, range), K/uL	8 (1, 24)	7 (0, 41)
Absolute reticulocyte counts (median, range), K/uL	**26.4 (0, 59.5)**	**17.9 (2.0, 97.1)**
Absolute lymphocyte counts (median, range), K/uL	1.5 (0.2, 4.3)	1.4 (0.1, 4.0)

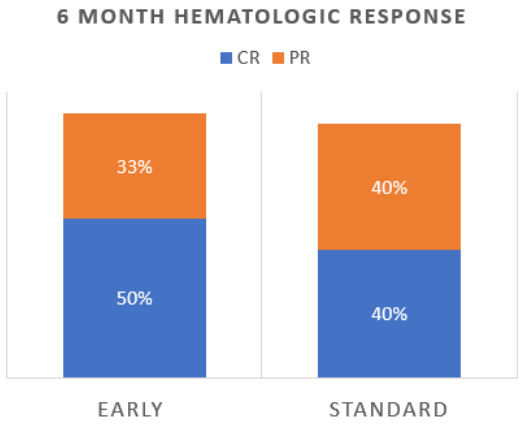
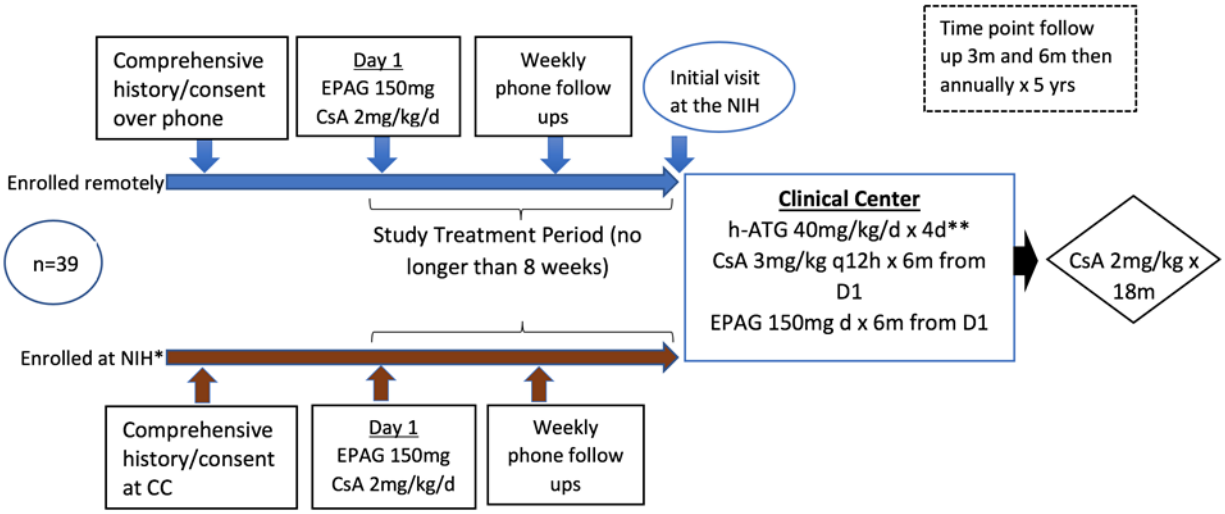


Figure 1: Comparison of 6 month responses

3 STUDY DESIGN

This study is designed as a non-randomized, pilot study in which subjects with SAA will be started with investigational early initiation of CsA and EPAG either before arrival to the NIH or at the NIH while work up is being completed. When the treatment is initiated remotely, it will be under oversight of investigators at NHLBI. At the CC, patients will receive standard treatment with higher dose CsA, EPAG, and h-ATG. h-ATG may be omitted if complete response is achieved.

Cohort 1 (original, n=39)



*D1 – Patients will be evaluated and enrolled at the NIH if able to arrive for initial visit within 72 hours of referral. If ANC <200 at the initial visit at NIH, treatment with all three drugs may be initiated without waiting for the cytogenetics.

**h-ATG may be omitted in patients who have achieved a complete response at the initial NIH visit after initiating oral treatment remotely

Note: Study treatment period of 8 weeks may be extended as defined in Section 9.6

Extension Cohort (n=41, same study design as above)

4 ELIGIBILITY ASSESSMENT

All subjects aged 3 and older with treatment naïve SAA and lack a suitable matched sibling marrow donor or are not allogeneic transplantation candidates due to patient choice, advanced age, or infeasibility of transplantation will be considered for enrollment. Treatment naïve is defined as not

having received a standard course of prior immunosuppressive therapy (ATG, cyclosporine, alemtuzumab, and high dose cyclophosphamide), or eltrombopag.

Eligibility may be determined on an NHLBI screening protocol (97-H-0041). The laboratory and clinical data obtained outside NIH may be accepted to determine eligibility. Subjects will be asked to sign a release of records to allow medical record review to obtain additional information about general and hematologic health. Bone marrow biopsy report (performed at outside facility) will be obtained and reviewed at the NIH and if there is any question about the diagnosis then the specimen will be obtained and confirmed prior to enrollment. The time between determination of eligibility and signing consent to participate on this protocol, and initiate treatment may not exceed 4 weeks. The time between initiating oral treatment to first visit at the Clinical Center may not exceed 8 weeks unless grace period is granted based on special circumstances (defined in Section 9.6). Due to the nature of SAA, counts may fluctuate depending on transfusions. Because of this, the lowest clinical laboratory result (absolute neutrophil count, platelet, and/or absolute reticulocyte count) obtained within 8 weeks prior to treatment initiation can be used for eligibility determination.

4.1 INCLUSION CRITERIA

1. Age ≥3 years old
2. Weight >12Kg
3. Severe aplastic anemia:

   Bone marrow cellularity <30% (excluding lymphocytes)

   AND

   At least two of the following:

- Absolute neutrophil count <500/μL
- Platelet count <20,000/μL
- Absolute reticulocyte count <60,000/μL

4.2 EXCLUSION CRITERIA

1. Known diagnosis or high suspicion of Fanconi anemia or other constitutional marrow failure syndrome
2. Evidence of a clonal disorder on cytogenetics performed within 12 weeks of study entry involving chromosome 7 or complex karyotype. Patient will not be excluded if cytogenetics are not done or are pending
3. A course of prior immunosuppressive therapy (ATG, cyclosporine, alemtuzumab, and high dose cyclophosphamide), or eltrombopag
4. SGOT or SGPT >2.5 times the upper limit of normal or total bilirubin >1.5 x upper limit of normal
5. Subjects with liver cirrhosis (as determined by the investigator).
6. Subjects with human immunodeficiency virus (HIV) who are not receiving antiretroviral therapy, have detectable HIV RNA viral load and have CD4 cell count <200/μL, or are on anti-retroviral therapy that interacts with the study drugs. subjects will not be excluded if HIV testing is pending or unavailable.
7. Glomerular filtration rate (GFR) <40 mL/min/1.73m^2
8. Hypersensitivity to EPAG or its components

9. Infection not adequately responding to appropriate therapy
10. Moribund status or concurrent hepatic, renal, cardiac, neurologic, pulmonary, infectious, or metabolic disease of such severity that it would preclude the patient's ability to tolerate protocol therapy, or that death within 7-10 days is likely
11. Potential subjects with cancer who are on active chemotherapeutic treatment or who take drugs with hematological effects will not be eligible
12. Inability to understand the investigational nature of the study or to give informed consent or does not have a legally authorized representative or surrogate that can provide informed consent per section 12.6.
13. Inability to swallow
14. Unable to participate in audio/video telecommunication
15. Inability to ship the study drug to participant
16. History or current diagnosis of cardiac disease indicating significant risk of safety for patients participating in the study such as uncontrolled or significant cardiac disease, including any of the following: Recent myocardial infarction (within last 6 months), uncontrolled congestive heart failure, unstable angina (within last 6 months), clinically significant (symptomatic) cardiac arrhythmias (e.g., sustained ventricular tachycardia, and clinically significant second or third degree AV block without a pacemaker.), long QT syndrome, family history of idiopathic sudden death, congenital long QT syndrome or additional risk factors for cardiac repolarization abnormality, as determined by the investigator.
17. Impaired cardiac function, such as: Corrected QTc >450 msec using Fridericia correction (QTcF) on the screening ECG (using triplicate ECGs), other clinically significant cardio-vascular disease (e.g., uncontrolled hypertension, history of labile hypertension), history of known structural abnormalities (e.g. cardiomyopathy).
18. Concurrent participation in an investigational study within 30 days prior to enrollment or within 5-half-lives of the investigational product, whichever is longer. Note: parallel enrollment in a disease registry is permitted.
19. Known thrombophilic risk factors. Exception: Subjects for whom the potential benefits of participating in the study outweigh the potential risks of thromboembolic events, as determined by the investigator.
20. Women of child-bearing potential, defined as all women physiologically capable of becoming pregnant, unless they are using basic methods of contraception during dosing of study treatment. Basic contraception methods include:
    - Total abstinence (when this is in line with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception
    - Female sterilization (have had surgical bilateral oophorectomy with or without hysterectomy), total hysterectomy, or tubal ligation at least six weeks before taking study treatment. In case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment
    - Male sterilization (at least 6 months prior to screening). The vasectomized male partner should be the sole partner for that subject
    - Barrier methods of contraception: Condom or Occlusive cap. For the UK: with spermicidal foam/gel/film/cream/ vaginal suppository

- Use of oral, injected or implanted hormonal methods of contraception or placement of an intrauterine device (IUD) or intrauterine system (IUS), or other forms of hormonal contraception that have comparable efficacy (failure rate <1%), for example hormone vaginal ring or transdermal hormone contraception.
    - In case of use of oral contraception women should have been stable on the same pill for a minimum of 3 months before taking study treatment.

21. Female subjects who are nursing or pregnant (positive serum or urine B-human chorionic gonadotrophin (B-hCG) pregnancy test) at screening or pre-dose on Day 1
22. Sexually active males unless they use a condom during intercourse while taking the drug during treatment, and for 7 days after stopping treatment (and for an additional 12 weeks [for genotoxic compounds]) and should not father a child in this period. A condom is required to be used also by vasectomized men as well as during intercourse with a male partner in order to prevent delivery of the drug via semen.

4.3 INCLUSION OF PREGNANT WOMEN, FETUSES OR NEONATES

The protocol does not intentionally enroll pregnant women due to unknown fetal risk with eltrombopag. However, if a patient gets pregnant during the trial participation period, she may remain on study for non-invasive safety and outcomes follow-up (see section 9.6 and 10.7.5 for details).

5 TREATMENT PLAN

Patients will commence therapy with cyclosporine and eltrombopag as outpatients (if transfer to the Clinical Center is anticipated to require >72 hours or upon evaluation to the Clinical Center if <72 hours). Medications will be shipped to the patient at their provided address. After beginning cyclosporine and eltrombopag, monitoring blood work will be obtained at least weekly, more frequent if clinically indicated. For outpatients, a member of the NIH bone marrow failure team (research nurse, physician assistant, or physician) will speak by telephone with the patient or responsible family member every week, to ascertain symptoms and for any evidence of drug toxicity or intolerance (Appendix B).Within 8 weeks after consent, all subjects will have their evaluation at the Clinical Center except if grace period is granted in special circumstances (defined in Section 9.6) and receive the standard treatment with h-ATG, higher dose cyclosporine (3mg/kg twice a day), and eltrombopag daily after full work up is completed here. For patients without grace period extension, the evaluation will take place within 8 weeks of initiation of oral treatment but the administration of the standard treatment may not occur within the 8 weeks' time frame. If ANC <200 at the initial visit at NIH, treatment with all three drugs may be initiated without waiting for the cytogenetics.

If participants are unable to come to the NIH due to unanticipated reason beyond their control, the administration of the standard treatment with h-ATG, higher dose cyclosporine, and continuation of eltrombopag daily may be performed by subject's local physician/institution with guidance from the NIH bone marrow failure team after the full work up is completed as listed in section 6.1 (Clinical studies to be performed at the Clinical Center on first visit) and results reviewed by the NIH bone marrow failure team. If this is to occur, specialized studies (marked as * in section 6.1) may be omitted.

5.1 CYCLOSPORINE (CSA)

Day 1 of cyclosporine to Day 1 of admission for h-ATG:

For all subjects, cyclosporine will be started on day 1 at 2mg/kg/day by mouth administered daily at a fixed dose until Day 1 of h-ATG. Given low dose, cyclosporine therapeutic trough levels will only be checked per PI or local physician's discretion if concerning toxicity signs or symptoms. Cyclosporine dose may be interrupted or adjusted as clinically indicated at the discretion of the investigator for side effects. Interruptions or dose adjustments as a consequence of a serious adverse event will be included in the SAE Report.

Cyclosporine can result in many common side effects including headaches, nausea, gastrointestinal upset, hypertension, electrolyte imbalances and kidney injury. A complete list of adverse events can be found in section 12.4.2. Additionally, Appendix A will be sent to the outside physician to serve as a guiding document about drug administration, drug interactions (listed in section 5.11), dosage, adverse events, and monitoring. Our research team will be conducting weekly telephone follow up with patients +/- physicians to manage any side effects related to medications.

Day 1 of h-ATG to month 6 dosing:

For subjects ≥ 12 years of age, cyclosporine will be increased to 3 mg/kg/dose by mouth administered every 12 hours (total daily dose of 6 mg/kg/day). Dosing will be based on actual body weight except in obese subjects. For obese subjects (defined as a body mass index > 35 in adult subjects [> 17 years of age, cyclosporine dosage will be based on an adjusted body weight that is calculated as the midpoint between the ideal body weight and actual body weight (see below for definition of ideal body weight). Pediatric patients (aged 17 and under) will dose on actual body weight for patients < 125% Ideal Body Weight (IBW). For those >125% IBW, dosing will be based on adjusted ideal body weight (see below).

For subjects < 12 years of age, cyclosporine will be increased to 6mg/kg/dose by mouth administered every 12 hours (total daily dose of 12 mg/kg/day). Patients aged 17 and under will dose on actual body weight for patients < 125% IBW. For those >125% IBW, dosing will be based on adjusted ideal body weight.

Cyclosporine dosing will be adjusted, at the investigator's discretion, to aim for a therapeutic trough level between 200 and 400 mcg/L. In subjects who had a therapeutic CsA level established prior to protocol enrollment, the same CsA dose will be initiated with the h-ATG and adjusted accordingly. Cyclosporine dose may be interrupted or adjusted as clinically indicated at the discretion of the investigator for side effects. Interruptions or dose adjustments are as the consequence of a serious adverse event, the interruption will be included in the SAE Report.

In between NIH landmark visits, only CsA levels will be tracked and recorded into the clinical database.

Ideal Body Weight Definitions:

Ideal body weight (adult male, age > 17): 50 kg + 2.3 kg per inch over 5 feet

Ideal body weight (adult female, age > 17): 45.5 kg + 2.3 kg per inch over 5 feet

Ideal body weight (pediatrics, ages 2–17):

a) < 5 feet tall:

IBW (kg) = [height (cm)]^2 x 1.65 / 1000

b) 5 feet and taller:

Male IBW (kg) = 39 + (2.27 x height in inches over 5 feet)

Female IBW (kg) = 42.2 + (2.27 x height in inches over 5 feet)

Pediatric patients (aged 17 and under) will dose on actual body weight for patients < 125% Ideal Body Weight (IBW). For those >125% IBW, will dose based on adjusted ideal body weight as follows:

Adjusted Ideal Body Weight = IBW + 0.25 (Actual weight - IBW). Month 6 to Month 24 cyclosporine dosing:

At the 6-month landmark visit, responders will have the CsA dose reduced to 2 mg/kg/day administered orally at a fixed dose through the 24 month timepoint as done in 12-H-0150 protocol.

In event of relapse during treatment period, cyclosporine dose will be increased to full dose per PI discretion. In absence of response to cyclosporine, eltrombopag re-initiation will be considered per PI discretion.

Medication dosing errors, dose delays or dosing interruptions:

Interruptions such as delays in request for medication refills or medication errors by subjects, or during circumstances such as COVID 19 pandemic that do not result in a serious adverse event or impact the integrity of the research data, will only be recorded in the medical record.

5.2 ELTROMBOPAG

Subjects will initiate EPAG at a starting daily dose as detailed in Table 3, according to age and ethnicity. Pediatric subjects between 12 and 17 years of age will receive the adult dose of 150 mg daily. Those between 6 and 11 will start at 75 mg, and children between 3 and 5 years of age will be started at 2.5 mg/kg (Table 1).

To adjust for the higher expected exposure in patients from East Asian and Southeast Asian ancestry, the starting dose for East Asian and Southeast Asian subjects between 12 and 85 years of age will be 75 mg once daily. For East Asian and Southeast Asian subjects between 6 and 11 years of age, the starting dose will be 37.5 mg once daily, and for children between 3 and 5, the starting dose will be 1.25 mg/kg (Table 3). EPAG will be administered orally as tablets or an oral suspension from sachet of EPAG powder. If an adult receives the oral suspension administration of EPAG, the required number of sachets will be used to administer the below adult doses. Detailed information about the supply and tablet/PfOS availability is in section 13.1.

Table 1. Dosing according to age and ethnicity

Age groups	Daily dose
Non-Asian	
12-85	150 mg
6-11	75 mg
3-5	2.5 mg/kg
East Asian, Southeast Asian	
12-85	75 mg
6-11	37.5 mg
3-5	1.25 mg/kg

EPAG may be taken on an empty stomach (1 hour before or 2 hours after a meal) or with food containing little (<50 mg) or preferably no calcium or dairy products. Allow at least a 4-hour interval between EPAG and other medications or products containing polyvalent cations (e.g. calcium, magnesium, aluminum, zinc, selenium or iron) such as antacids, dairy products, and mineral supplements to avoid significant (70-75%) reduction in eltrombopag absorption due to chelation.

EPAG dose may be interrupted when clinically indicated at the discretion of the investigator (for example if the patient is in the intensive care unit and unable to take PO medications).

Interruptions such as delays in request for medication refills or medication errors by subjects, or during circumstances such as COVID 19 pandemic that do not result in a serious adverse event or impact the integrity of the research data, will only be recorded in the medical record.

5.3 DOSE ADJUSTMENTS OF ELTROMBOPAG

The daily dose of eltrombopag will be decreased according to the following rules:

Platelet Count	Dose Adjustment or Response
>200,000/μL (untransfused) at any time on study	Decrease dosage by 25mg every 2 weeks to lowest dosage that maintains platelet count ≥ 50,000/μL. In children under 12, the dose will be decreased by 12.5 mg.
>400,000/μL (untransfused) at any time on study	Discontinue eltrombopag for one week, if platelets fall to <200,000/μL; restart

Platelet Count	Dose Adjustment or Response
	at dosage decreased by 25 mg/day (or 12.5 mg in children under 12).

5.4 Dose Delays, Modifications or Discontinuation of Eltrombopag for Non-Hematologic Side Effects

5.4.1 Infection

Subjects who experience an infection requiring intravenous antibiotics will not have EPAG discontinued. If the subject experiences infection severe enough to require vasopressors or intubation, the drug will be withheld until the subject is stable.

5.4.2 Liver function abnormalities

Eltrombopag dose may be interrupted when clinically indicated at the discretion of the investigator. Recommend dose modifications for isolated ALT or AST elevation according to the following rules:

Isolated ALT or AST elevation	Dose modification
>ULN – 3.0 x ULN	Maintain dose level
>3.0 – 5.0 x ULN	Maintain dose level. Repeat liver function tests as soon as possible, preferably within 48-72 hours, from awareness of the abnormal results. If abnormal laboratory values are confirmed upon the repeat test, monitor liver function tests weekly or more frequently if clinically indicated until resolved to **≤3.0 x ULN.**
>5.0 – 10.0 x ULN	Interrupt dose. Repeat liver function tests as soon as possible; preferably within 48-72 hours from awareness of the abnormal results. Monitor liver function tests weekly, or more frequently if clinically indicated until resolved to **≤3.0 x ULN then:** If resolved in ≤14 days, maintain dose level. If resolved >14 days, decrease one dose level
>10.0-20.0 x ULN	Interrupt dose. Repeat liver function tests as soon as possible, preferably within 48-72 hours from awareness of the abnormal results. Monitor liver function tests weekly, or more frequently if clinically indicated until resolved to **baseline.** Then decrease one dose level.
>20.0 ULN	Discontinue subject from study drug treatment. Repeat liver function tests as soon as possible, preferably within 48-72 hours from awareness

Isolated ALT or AST elevation	Dose modification
	of the abnormal results. Monitor liver function tests weekly or more frequently if clinically indicated until resolved to **baseline or stabilization over 4 weeks.**

5.4.3 Skin rash

If the subject experiences diffuse erythematous skin rash that appears to be drug related and no other drugs are implicated, EPAG will be discontinued.

5.4.4 Dose modifications/discontinuation for other reasons

Study drug dosing is to be temporarily discontinued in subjects unable to ingest the drug due to mucositis, persistent vomiting, or any other reasons during which patient is unable to take drug by mouth.

Early discontinuation of treatment

Eltrombopag must be permanently discontinued if any of the following events occur or is identified at any time during the study:

1. Development of myelodysplastic syndrome, acute myeloid leukemia, or cytogenetic clonal evolution to chromosome 7 or complex karyotype
2. Positive pregnancy test at any time during the study
3. Difficulties to continue the study treatment due to treatment related AE(s)
4. Subject is found to be significantly non-compliant with the requirements of the protocol (including treatment non-compliance)

5.5 Dose Delays, Modifications or Discontinuation of Eltrombopag for Hematologic Side Effects

5.5.1 Thrombosis/Embolism

Subjects who experience a deep venous thrombosis (other than a line-related upper extremity thrombosis) or a pulmonary embolus, a TIA or stroke, or a myocardial infarction at any time while on EPAG, it will be discontinued but CsA and hATG will be continued. Subjects with platelet counts of > 50,000/μL at the time of thrombosis will be treated with enoxaparin or another appropriate anticoagulant as clinically indicated unless the platelet count drops below 20,000/μL or they complete a standard 3-6 months course of anticoagulation.

5.6 Horse Anti-thymocyte-Globulin (h-ATG) Administration

A single treatment course of h-ATG will be administered at a dose of 40 mg/kg/day for 4 days. h-ATG will be administered after the patient establishes care here at the Clinical Center and all required tests are performed. Dose will be calculated based on actual body weight. h-ATG will be infused intravenously for approximately 4 hours daily. The infusion time may vary based on the patient and circumstances. Infusion times may be extended up to 24 hours to improve tolerance of infusion related side effects such as fever, chills and hypotension if necessary.

5.7 Pre-medications and Management of Infusion Reactions

Subjects will receive pre-medication approximately 30 minutes prior to infusion of ATG as follows:

- oral diphenhydramine 1 - 1.5 mg/kg/dose (NTE 50 mg) administered orally or intravenously, and;
- oral acetaminophen 10-15 mg/kg/dose (NTE 650 mg)

Serum sickness prophylaxis with oral prednisone at 1 mg/kg/d will begin prior to the first dose of h-ATG, and will be continued for 10 days total and then tapered over the subsequent 7-14 days. Methylprednisone at equivalent doses may be substituted for prednisone. Those subjects who develop serum sickness may require a longer tapering schedule and will be dosed individually as clinically indicated. Infusion reactions will be treated symptomatically (e.g., antiemetics, IV fluid hydration, acetaminophen, antihistamines, inhaled bronchodilators, meperidine). Prednisone dose will be calculated based on actual body weight.

In case of moderate or severe reactions hydrocortisone will be given and the infusion will be discontinued and restarted at a slower rate once the symptoms have subsided. If a subject has a persistent severe infusion reaction that does not respond to measures to ameliorate the signs/symptoms associated with the infusion, the h-ATG infusion will be discontinued (see section off study criteria) and subjects will go off study.

5.8 SUPPORTIVE CARE

- Transfusion support (blood and platelets) as clinically indicated.
- Hematopoietic growth factors (e.g., G-CSF, GM-CSF, or erythropoietin) if deemed necessary by the investigator or treating physician. Romiplostim (N-Plate) or IL-11 (Neumega) should not be administered.
- Estrogen, combination OCP's, or leuprolide as indicated for uterine bleeding (menses suppression).

5.9 CONCURRENT MEDICATIONS

Cyclosporine/magnesium: Subjects on CsA are expected to have magnesium wasting as a common side effect as long as EPAG is administered 4 hours after oral magnesium. Magnesium supplementation will not be given concurrently with EPAG as it may interfere with EPAG's absorption. The drug-drug interaction potential between EPAG and CsA is unknown. Both CsA and EPAG are inhibitors of OATP and BCRP drug transporters, and EPAG is a substrate of BCRP in vitro. It is not known if the combination will result in any PK changes to either drug. Subjects will be monitored for signs of CsA toxicity during the study, and therapeutic drug monitoring can be instituted as required. In the event of liver function abnormalities as a consequence of a drug-drug interaction between EPAG and CsA, EPAG will be dose-reduced according to section 5.4.

Inhibitors of cytochrome p450: In vitro studies demonstrate that CYP1A2 and CYP2C8 are involved in the oxidative metabolism of eltrombopag. Trimethoprim, gemfibrozil, ciprofloxacin, fluvoxamine and other moderate or strong inhibitors of CYPs may therefore theoretically result enhanced activity of EPAG, however these interactions have not yet been established in clinical studies. All subjects on cyclosporine require prophylaxis against PCP and will be given inhaled pentamidine instead of Trimethoprim/sulfamethoxazole (TMP/SULF).

NIH SAA patients are routinely placed on pentamidine instead of TMP/SULF for PCP prophylaxis to avoid potential marrow-suppressive effects of TMP/SULF anyway. Subjects aged 5 years and over will receive pentamidine for PCP prophylaxis but children under 5 years of age (approximate) are often not able to complete the inhalation treatment with pentamidine and will receive dapsone or another prophylactic regimen. Other CYP inhibitors can be used concomitantly but with careful attention to possible increased EPAG activity and toxicity.

Other medications: Subjects may continue on any of the medications that they were prescribed prior to study enrollment for co-morbid conditions, with the exception of N-Plate and Neumega (See Section 5.8).

5.10 Infection Prophylaxis and Monitoring

Pneumocystis jiroveci **prophylaxis:** During lower dose of CsA, routine Pneumocystis jiroveci phrophylaxis (PJP) is not indicated. However, in patients with severe lymphopenia (Absolute lymphocyte counts <500/uL), PJP prophylaxis will be considered per PI discretion. It will be initiated upon starting h-ATG and higher dose of cyclosporine 3mg/kg q12h (6mg/kg q12h for pediatric) at the Clinical Center. Aerosolized pentamidine will be used as prophylaxis against *Pneumocystis jiroveci,* 300 mg approximately every 4 weeks by inhalation beginning the first month of higher cyclosporine therapy and to continue until month 6 when the cyclosporine dose is reduced.

Dapsone or another prophylactic regimen against *Pneumocystis jiroveci* may be substituted at the discretion of the PI. Bactrim (TMP/SULF) will be avoided because trimethoprim is a moderate to strong inhibitor of CYPs that may theoretically result in enhanced activity of EPAG. Children under 5 years of age (approximate) are often not able to complete the inhalation treatment with pentamidine and will receive dapsone or another prophylactic regimen at the discretion of the PI.

Antiviral prophylaxis: During lower dose of CsA, Valcyclovir is not indicated. However, in patients with severe lymphopenia (Absolute lymphocyte counts <500/uL), Valacyclovir prophylaxis will be considered per PI discretion. Valacyclovir, 500 mg once daily, will be administered for at least 1 month in all subjects regardless of HSV serology status at the time of h-ATG and higher dose cyclosporine at the Clinical Center. Pediatric subjects less than 40 kg will receive acyclovir (or equivalent) at 20mg/kg PO q12h to a maximum dose of 800mg q12h. Prophylaxis may be extended at the discretion of the PI.

Antibacterial and antifungal prophylaxis will not be included systematically with the immunosuppressive regimen but may be administered at the discretion of the PI or treating physician on a case-by-case basis.

5.11 Instructions to Subjects

Special instructions regarding CsA:

Regarding concomitant medications: Certain medications can change the level of cyclosporine in the blood. Some of these medications are erythromycin, ketoconazole, diltiazem, rifampin, phenytoin and phenobarbital. We will ask subjects to inform us of any medication taking concomitantly while on the study.

Regarding prohibited foods: Grapefruit and grapefruit juice may increase the effects of CsA by increasing the amount of this medicine in the body. Subjects will be advised not to eat grapefruit or drink grapefruit juice while taking this medicine.

Regarding Immunizations: While taking CsA and for at least three months following discontinuation, immunizations should be avoided, and any planned immunization should be discussed with study investigators. There is almost no possibility that a vaccination given during this time period will be effective in stimulating immunity. Any live or attenuated vaccine may result in an infection, due to compromised immunity on CsA and h-ATG. Subjects should also avoid close household contact with individuals receiving the live oral polio vaccine for at least 72 hours following administration.

Special instructions regarding eltrombopag:

Timing in relation to food: Subjects will be advised to take EPAG on an empty stomach (1 hour before or 2 hours after a meal), or adhere to a restricted diet of low calcium (dairy products) and polyvalent cations at least 4 hours apart from co-administration of EPAG.

Timing in relation to antacids and polyvalent cations: Because co-administration of EPAG with antacids decreased plasma AUC of EPAG by 70%, subjects will be advised to take the EPAG at least 4 hours apart from antacids and other products containing polyvalent cations (i.e. aluminum, calcium, magnesium, iron, selenium and zinc) such as mineral supplements and dairy products.

5.12 Complications

Due to severely low blood counts in this disease process, there are several complications that may require urgent care/hospitalizations. Patients may receive this care at any hospital/facility that has capabilities of treating the presenting illness. Our research team will remain engaged in diagnostic/treatment decisions. Patients may be transferred to the Clinical Center if the treating hospital/facility lacks necessary treatment.

Common reasons for hospitalization in SAA patients may include bleeding, infections, and anemia related symptoms. Common reasons for hospitalization for the study treatment may include malignant hypertension/posterior reversible encephalopathy syndrome, hepatotoxicity, and acute renal failure.

6 CLINICAL MONITORING

Once patient has arrived at the Clinical Center, samples will be ordered and tracked through the CRIS Screens. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical record.

6.1 PRE-STUDY EVALUATION/SCREENING

The following general screening procedures will be done either remotely during the initial telephone interview based on the labs done outside of NIH or at the NIH for those that are able to arrive at the NIH <72 hours.

- Medical history
- Physical examination (will be confirmed with the referring hematologist)
- Family history
- Concurrent medications

PI may accept results from labs done outside of NIH as both screening and baseline as long as they are done within 30 days of enrollment, bone marrow aspirate/biopsy results will be accepted as long as it is performed within 90 days of enrollment:

- Complete blood count with differential
- Reticulocyte count
- Type and screen
- Acute care (Na, K, Cl, CO2, creatinine, glucose, and urea nitrogen), Hepatic (Alk Phosphatase, ALT, AST, and Total Bilirubin), and lactate dehydrogenase. Direct bilirubin will be obtained per PI discretion if total bilirubin is elevated.
- Pregnancy test (urine or blood HCG in women of childbearing potential)
- Bone marrow aspiration and core biopsy stained for standard morphologic analysis and quantitation of cellularity with hematoxylin and eosin. Marrow sample will be reviewed here at the NIH with our pathologist.
- HIV serology (optional per PI discretion based on the clinical history)

Clinical studies to be performed at the Clinical Center on **first visit**:

- Complete blood count with differential and reticulocyte count
- Type and screen
- Acute care (Na, K, Cl, CO2, creatinine, glucose, and urea nitrogen), Mineral (Phosphorus, Magnesium, Albumin, and Calcium), Hepatic (Alk Phosphatase, ALT, AST, Total Bilirubin), and Other (Total Protein, Uric Acid, and LDH). Direct bilirubin will be obtained per PI discretion if total bilirubin is elevated.
- Thyroid function tests, Folate, B12 level, iron panel (ferritin, transferrin, % saturation), DAT (direct antiglobulin test)
- Viral serologies for hepatitis A, B, C, HIV, HSV, EBV, and CMV (if not already available)
- PPD (at risk subjects only, based on history or geography) per PI discretion
- Flow cytometry of the peripheral blood to quantitate GPI-negative cells
- EBV and CMV PCR (if not already available)*
- HLA typing (if not already available)*
- Telomere length of leukocytes (if not already available)*
- Diepoxybutane (DEB) testing for Fanconi anemia per PI discretion (if not already available)*
- Lymphocyte peripheral blood phenotyping (analysis of T, B, and NK subsets via flow cytometry)*
- Bone marrow aspiration and core biopsy to be stained for standard morphologic analysis and quantitation of cellularity with hematoxylin and eosin, and special stains to assess reticulin and collagen, primitive stem and progenitor cells via CD34 immunohistochemistry, and other lineage-specific or special stains as indicated to classify any abnormalities**
- Bone marrow chromosomal analysis via standard cytogenetic techniques**
- EKG (to be done prior to h-ATG)
- Placement of a central line if subject does not have a pre-existing indwelling central venous catheter if treatment with h-ATG is indicated

- Chest X-ray - (not required to be performed, per PI discretion))

  * Results if performed within the last 180 days either at NIH or outside NIH may be accepted. Only repeated if there are changes in the clinical status that may impact the results.
  ** At this visit, up to 30 mL total including the 15ml for exploratory/correlative research sample may be collected. For minors, the bone marrow biopsy & aspirate procedure will be done with general anesthesia.

6.2 On Study Off Site Monitoring, prior to initial Clinical Center visit if enrolled on study remotely

Prior to establishing care at the Clinical Center, patient will be followed closely by his/her local physician under complete guidance of the team at the Clinical Center (Appendix B). Weekly (+/- 3 days) telephone follow-up will be established with the patient. All the progress notes and labs indicated below will be requested to be sent from home physician to the research nurse. These documents will serve as source documents to collect data.

If a patient is unable to participate in a weekly follow up due to acute illness and hospitalization, the team will make contact with the treating physician and document patient's condition in lieu of a telephone follow up.

- Every 2-4 weeks – Progress note including history and physical examination by local physician
- Complete blood counts at least weekly (+/- 3 days), more frequent if clinically indicated. Frequency may be decreased to once every 2 weeks (+/- 4 days) per PI discretion. Only the following parameters will be recorded in the clinical database because of their relevance to the underlying disease and study treatment: absolute lymphocyte count (ALC) or lymphocyte percentage, absolute neutrophil count (ANC), or neutrophil percentage and white blood cell count if an absolute neutrophil count is not provided, hemoglobin, and platelets. Absolute reticulocyte count (or reticulocyte percentage and red blood cells [RBC] if an absolute reticulocyte count is not provided) will be recorded if available.
- Hepatic and renal panels (home panels must include hepatic transaminases, urea nitrogen (BUN), serum creatinine, and total bilirubin) at least weekly (+/- 3 days), more frequent if clinically indicated. Frequency may be decreased to once every 2 weeks (+/- 4 days) per PI discretion.
- Only the following parameters will be recorded in the clinical database because of their relevance to the underlying disease and study treatment: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, creatinine, blood urea nitrogen (BUN), glucose, sodium, potassium, CO2 and chloride. Direct bilirubin will be obtained per PI discretion if total bilirubin is elevated.
- CsA blood level will only be obtained per PI/local physician discretion as clinically indicated. Due to low dose, there is no therapeutic target.

6.3 On Study Monitoring, Day 1 of h-ATG Through Hospital Discharge

On treatment monitoring will consist of the following, unless the hospitalization is interrupted due to the participant leaving the hospital "on pass" but not to exceed more than 7 days:

- Pregnancy test (urine or blood HCG in women of childbearing potential) does not need to be repeated if done within two weeks of initiation of h-ATG unless clinically indicated
- CBC with differential (daily)*
- Acute care, Mineral, and Hepatic (every other day)*
- Reticulocyte count (weekly +/- 3 days)*
- Vital signs (daily)
- CsA blood level will be monitored every week (+/- 3 days) while inpatient in the hospital and continued per section 6.4. The blood level will be monitored to ensure therapeutic range of 200 – 400 ng/ml is achieved. CsA dosage will be adjusted to target this range unless otherwise indicated by PI due to toxicities or intolerance. More frequent drug serum levels may be obtained as needed to achieve target therapeutic levels and avoid toxicity.

*In case of prolonged hospitalization (beyond 14 days), the marked labs will be required no more than twice a week.

6.4 On Study Monitoring (hospital discharge through 6 months)

After completing h-ATG administration, subjects will remain hospitalized until clinically stable. Post-discharge, subjects may be followed by their home physician or at the Clinical Center. Progress notes and laboratory results from home physicians will be faxed to the research nurse. Standard of care tests will be done as needed and may include the tests listed below. Changes in frequency of the tests below will be performed as clinically indicated at the investigator's discretion.

- Complete blood counts with differential every 1-2 weeks (+/-4 days). Only the following parameters will be recorded in the clinical database because of their relevance to the underlying disease and study treatment: absolute lymphocyte count (ALC) or lymphocyte percentage, absolute neutrophil count (ANC), or neutrophil percentage and white blood cell count if an absolute neutrophil count is not provided, hemoglobin, platelets, and absolute reticulocyte count (or reticulocyte percentage and red blood cells [RBC] if an absolute reticulocyte count is not provided).
- Hepatic and renal panels (home panels must include hepatic transaminases, urea nitrogen (BUN), serum creatinine, and total bilirubin) every 1-2 weeks (+/- 4 days). Magnesium may be requested in patients with hypomagnesemia per PI.

  Only the following parameters will be recorded in the clinical database because of their relevance to the underlying disease and study treatment: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, creatinine, blood urea nitrogen (BUN), glucose, sodium, potassium, CO2 and chloride. Direct bilirubin will be obtained per PI discretion if total bilirubin is elevated.
- CsA blood levels will be monitored every week (+/- 3 days) for the first month and then every 2 weeks (+/- 4 days) for the remainder of the treatment period once levels are stabilized in the therapeutic range of 200 – 400 ng/ml. CsA dosage will be adjusted to

target this range unless participants cannot tolerate therapeutic dosing of CsA. More frequent drug serum levels may be obtained as needed to achieve target therapeutic levels and avoid toxicity, and conversely less frequent levels may be obtained as needed in individuals unable to tolerate therapeutic dosing of CsA. The dose may not necessarily be adjusted following every out of range CsA level; the decision to adjust the dose will be determined by the clinician judgement based on multiple data sources, that could include but not limited to time of measurement, prior trend of CsA levels, estimation of steady state, concomitant medications that may affect CsA metabolism, and renal function. CsA monitoring will be discontinued at 6 months.

Landmark 1, 2, 3 and 6-month monitoring

Subjects must be evaluated at the NIH Clinical Center at the 6-month (+/-10 days) time point. 1 and 2-month (+/- 10 days) landmark time points are for evaluation of early hematological response only; therefore, do not require a visit at the Clinical Center. Per PI discretion, the 3 month time point visit may be conducted with subjects' local physician during special circumstances, where the risk of evaluation at the CC may outweigh the benefit. Tests with * below can be omitted from 3 month evaluation if the visit is being conducted with home physician. In addition, a tele/video meeting with the patient will be conducted by the bone marrow failure team member.

1- and 2-month

- Complete blood counts with differential
- Reticulocyte count

3- month and 6- month

- History and physical examination
- Complete blood counts with differential
- Acute care (Na, K, Cl, CO2, creatinine, glucose, and urea nitrogen), Mineral (Phosphorus, Magnesium, Albumin, and Calcium), Hepatic (Alk Phosphatase, ALT, AST, Total Bilirubin), and Other (Total Protein, Uric Acid, and LDH). Direct bilirubin will be obtained per PI discretion if total bilirubin is elevated.
- Reticulocyte count and other (cyclosporine level, ferritin, iron, transferrin, lactate dehydrogenase, protein)
- Urine pregnancy test (woman of childbearing age only)
- Bone marrow aspiration and core biopsy, to be stained for standard morphologic analysis and quantitation of cellularity with hematoxylin and eosin, and special stains to assess reticulin and collagen, primitive stem and progenitor cells via CD34 immunohistochemistry, and other lineage-specific or special stains as indicated to classify any abnormalities**
- Bone marrow chromosomal analysis via standard cytogenetic techniques**
- Flow cytometry of the peripheral blood to quantitate GPI-negative cells
- Lymphocyte peripheral blood phenotyping (analysis of T, B, and NK subsets via flow cytometry)*
- EKG will be performed at 3 months
- Transfusion records to be obtained including number of transfusions since last landmark visit as well as date of last transfusion

** At each of these visits, up to 30 mL total including the sample for research may be collected. Bone marrow aspiration, biopsy, and chromosomal analysis may be omitted if one was performed within 4 weeks of the timepoint analysis.

6.5 LONG TERM FOLLOW UP (6 MONTHS TO 5 YEARS)

After the 6-month visit, subjects must be evaluated at the Clinical Center at 12 months (+/- 30 days) and then yearly thereafter to 5 years (+/- 90 days) except in special circumstances (see below). Subjects will be seen by their home physician as clinically indicated and the Hematology Branch investigators and home physicians will remain in communication. Home physicians will monitor blood counts and other clinical parameters as clinically indicated. The following tests will be performed at each Clinical Center visit. At the clinical investigator's discretion, participants may be evaluated more frequently if medically indicated based on disease status.

PI discretion, during special circumstances (such as COVID 19 outbreak) where the risk of evaluation at the CC may outweigh the benefit, a tele/video visit may be conducted and test results received from subjects' local physician may be used. Tests with * below can also be omitted.

- History and physical examination
- Complete blood counts with differential
- Acute care (Na, K, Cl, CO2, creatinine, glucose, and urea nitrogen), Mineral (Phosphorus, Magnesium, Albumin, and Calcium), Hepatic (Alk Phosphatase, ALT, AST, Total Bilirubin), and Other (Total Protein, Uric Acid, and LDH). Direct bilirubin will be obtained per PI discretion if total bilirubin is elevated
- Reticulocyte count and other (ferritin, iron, transferrin, lactate dehydrogenase, protein)
- Urine pregnancy test (woman of childbearing age only)
- Bone marrow aspiration and core biopsy, to be stained for standard morphologic analysis and quantitation of cellularity with hematoxylin and eosin, and special stains to assess reticulin and collagen, primitive stem and progenitor cells via CD34 immunohistochemistry, and other lineage-specific or special stains as indicated to classify any abnormalities (optional for year 3, 4 and 5 depending on the peripheral blood counts and concern for clonal evolution)*
- Bone marrow chromosomal analysis via standard cytogenetic techniques (optional for year 3, 4 and 5 depending on the peripheral blood counts and concern for clonal evolution)*
- Flow cytometry of the peripheral blood to quantitate GPI-negative cells
- Lymphocyte peripheral blood phenotyping (analysis of T, B, and NK subsets via flow cytometry)
- Date of last transfusions (both blood and platelets) to be recorded in database since landmark visit

*At these visits, up to 30 mL total including the 15ml for exploratory/correlative research sample may be collected. For minors, the bone marrow biopsy & aspirate procedure will be done with general anesthesia.

6.6 GENETIC AND OTHER TESTS (MAY BE PERFORMED IN SOME PATIENTS)

- Testing for acquired somatic mutations in genes linked to leukemia and bone marrow

failure in a CLIA-certified commercial, and flow cytometry may be performed.

- Presence of mutations in genes known to be associated with inherited bone marrow failure disorders and/or telomere length in a CLIA-certified laboratory. When novel mutations are found, the respective samples may be further evaluated.
- Diepoxybutane stimulation testing of peripheral blood may be performed in subjects with cytopenias under the age of 40 or if clinical history or characteristics suggest Fanconi anemia.

6.7 RETURN OF RESEARCH RESULTS

For tests performed at the Clinical Center, if there are not expected (incidental) research test results relevant to subjects' disease (SAA) and can put them at high risk of other disease, we will provide subjects with the test results and the appropriate follow-up information.

6.8 EXTENDED ACCESS FOR RELAPSE

Indicated below are the procedures that can be performed on subjects that re-start oral drugs (cyclosporine, eltrombopag or both) due to relapse. At the clinical investigator's discretion, participants may be evaluated as medically indicated based on disease status. The procedures may be performed by the subjects' home physician or at the Clinical Center. If testing is done by home physician, progress notes and laboratory results from home physician will be sent to the research team. Subjects will be seen at the Clinical Center as clinically indicated while on extended access for relapse, but no less than annually until 5 years. Below is the list of procedures that may be performed at the clinical center visit at least once per year (+/- 90 days) or more frequently as medically indicated.

Procedures that may be performed when drugs are re-started:

- History and physical examination
- Pregnancy test (urine or blood HCG in women of childbearing potential) – Optional
- Complete blood counts with differential
- Reticulocyte count
- Chemistry panel (NIH Acute care, Mineral, Hepatic and Other panel, home laboratory chemistry panel must include electrolytes, hepatic transaminases, urea nitrogen (BUN), serum creatinine, total bilirubin, and reticulocyte count)
- CsA blood level will be monitored until levels are stabilized in the therapeutic range of 200 – 400 ng/ml. CsA dosage will be adjusted to target this range. More frequent drug serum levels may be obtained as needed to achieve target therapeutic levels and avoid toxicity. This test is only required if patient is on therapeutic levels of cyclosporine.
- Bone marrow aspiration and core biopsy, to be stained for standard morphologic analysis and quantitation of cellularity with hematoxylin and eosin, and special stains to assess reticulin and collagen, primitive stem and progenitor cells via CD34 immunohistochemistry, and other lineage-specific or special stains as indicated to classify any abnormalities (optional for year 3,4, and 5 based on the peripheral blood counts and concern for clonal evolution)*
- Bone marrow chromosomal analysis via standard cytogenetic techniques (optional for year 3,4, and 5 based on the peripheral blood counts and concern for clonal evolution)*
- Flow cytometry of the peripheral blood to quantitate GPI-negative cells

- Lymphocyte peripheral blood phenotyping (analysis of T, B, and NK subsets via flow cytometry)

*At these visits, up to 30 mL total including the 15ml for exploratory/correlative research sample may be collected. For minors, the bone marrow biopsy & aspirate procedure will be done with general anesthesia.

6.9 COVID-19

The impact of COVID-19 infection on SAA patients with marked neutropenia and with immunosuppressive treatment is currently unknown. Known risk factors include high age, and comorbidities particularly heart and lung disease. Data is limited and conflicting regarding the risks in immunosuppressed patients. Following treatment guidelines are made with current available information and may be amended with future evidence. Patients will be monitored with clinical vigilance and expedited testing in suspected cases.

- All patients will be educated on preventative practices.
- PCR test for SARS-CoV2 and respiratory viral panel will be performed in patients with fever, upper or lower respiratory tract symptoms.
- In patients with mild COVID-19 infection, lower dose CSA and EPAG treatment will not be delayed.
- In patients with severe COVID-19 infection, lower dose CSA and EPAG treatment may be delayed per PI discretion.
- Standard of care treatment (h-ATG, higher dose CSA and EPAG), which is more immunosuppressive than lower dose CSA, will be delayed at least 2 weeks from the diagnosis of COVID-19 and may be longer in severe cases based on their clinical condition.

7 CRITERIA FOR RESPONSE/CLONAL EVOLUTION/RELAPSE

Misdiagnosis is defined as diagnosis of other hematological or non-hematological condition to explain peripheral pancytopenia.

Non-compliance for feasibility is defined as not following up at the Clinical Center by 8 weeks (or within allowed grace period in special circumstances defined in section 9.6) or failure to comply with the recommended oral treatment or clinical/laboratory assessment.

Response is defined as blood counts no longer meeting the standard ("Camitta") criteria for severe pancytopenia in SAA (Section 4.1), equivalent to 2 of the following values obtained on 2 serial blood count measurements at least one week apart at landmark time points (2, 3, and 6 months).

- Absolute neutrophil count ≥500/ μL
- Platelet count ≥ 20,000/ μL
- Reticulocyte count ≥ 60,000/ μL

A **complete response (CR)** will be defined as (all 3 must be met):

- Absolute neutrophil count ≥1,000/ μL
- Platelet count ≥100,000/ μL
- Hgb≥10 g/dL

A **partial response (PR)** will be defined as blood counts that do not meet criteria for severe pancytopenia but are not sufficient for a CR.

Improvement in counts that are dependent upon exogenously administered growth factors or transfusion will not be considered as fulfilling response criteria.

Clonal Evolution: The presence of evolution to PNH will be defined by flow cytometric detection of > 1% GPI-deficient neutrophils at baseline and landmark time points through 5 years (only the ones where patient is evaluated at the Clinical Center, excludes months 1 and 2). Evolution to hyperproliferative/dysplastic myelodysplasia and/or acute leukemia will be assessed at landmark time points, or as clinically indicated between landmarks by examination of peripheral blood and bone marrow based on diagnosis and classification according to the WHO 2016 criteria. Evolution to clonal hematopoiesis will be defined by detection of new bone marrow cytogenetic abnormalities at landmark time points.

Relapse:

In this protocol, relapse is defined as having, EITHER,

1. Blood count decline to a degree where they again meet "Camitta" criteria (see Section 4.1) for severe aplastic anemia determined from at least 2 blood count measurements done within 14 days, or
2. Progressive decline in one or more blood counts, not explained by another clinical process (e.g. acute infection or medication related) determined from at least 2 blood count measurements done within 14 days.

The date of relapse will be recorded into the clinical database as the date the participant resumed immunosuppression with CsA or required higher doses of CsA if on the low dose prior to the 2-year landmark visit, resumed EPAG or h-ATG (if subject had not received h-ATG previously), or proceeded with another treatment for SAA.

A description of how the clinical assessment was made by the PI will be recorded in the clinical database. To corroborate the relapse, the most recent blood counts collected prior to the date of relapse will be recorded into the clinical database with the following parameters: absolute neutrophil count, or neutrophil percentage and white blood cell count if ANC is not available, hemoglobin, absolute reticulocyte count (or reticulocyte percentage and RBC if absolute reticulocyte count is not available) and platelets.

8 EXPLORATORY LABORATORY RESEARCH STUDIES

Intended use: During the course of participating on this study, additional 60 cc of blood (3 ml/kg not to exceed 60 ml of blood for pediatric subjects) at first visit to the Clinical Center and at landmark visits at 3, 6, 12 months and annually thereafter, and 15 ml of bone marrow aspirate (prior to ATG, 3 months, 6 months, 12 months, and annually thereafter) may be obtained for correlative laboratory research studies. Up to an additional 10 cc of blood may be collected during follow up every 1-6 months (according to how often they get their counts checked) for correlative laboratory studies. The protocol will cover shipment materials/costs for these additional samples to be returned to the NIH since they are for research use (no results given). Initial samples collected upon first visit at the Clinical Center may be obtained on another protocol, such as 04-H-0012. These studies are not used in assessing the primary endpoint but

are undertaken as descriptive or exploratory ancillary studies as listed below. Some or all may be performed on each subject, and they may be correlated with response.

- Thrombopoietin level
- CD34 cell number in whole blood and bone marrow aspirate by flow cytometry
- T cell receptor V-beta profile in the marrow and peripheral blood
- Extended peripheral blood flow cytometric phenotyping for cell surface or intracellular proteins
- Evaluation for the presence of abnormalities of the telomere repair complex including telomere length, DEB testing for Fanconi anemia.
- Evaluation for the presence of abnormalities of genes associated with hematopoiesis, via genetic testing or gene expression analysis.
- Evaluation for the presence of abnormalities of genes associated with inherited bone marrow failure or inborn errors of immunity.
- Serum cytokine, chemokines and soluble receptor levels
- Serum (or plasma) and cells for viral analyses
- Hematopoietic progenitor colony, long term-culture-initiating cell, and immunodeficient mouse engraftment assays for primitive cell content and function
- Single cell RNAseq or multi-omics on bone marrow specimens to detect chromosomal aneuploidy before and after treatment.
- Telomere length on peripheral blood leukocytes by flow-FISH before and after treatment

9 BIOSTATISTICAL CONSIDERATIONS

9.1 PRIMARY ENDPOINTS

The primary endpoint of this pilot study is to evaluate the safety and feasibility of rapid initiation of oral treatment regimen, CsA + EPAG prior to receiving standard regimen at the Clinical Center in treatment naïve SAA as a composite measure of the following:

- Rates of TRSAE (treatment related serious adverse events) compared to standard regimen (protocol 12-H-0150). TRSAE are defined in section 9.5.
- Rates of mis- and altered diagnoses compared to standard regimen. **Misdiagnosis** is defined as diagnosis of other hematological or non-hematological condition to explain peripheral pancytopenia such as myeloid neoplasm.
- Number and proportion of patients who are unable to comply with the outpatient early drug regimen and withdraw from study or who do not present to the Clinical Center for evaluation within 8 weeks (or within allowed grace period in special circumstances defined in section 9.6)

9.2 SECONDARY ENDPOINTS

Secondary endpoints will be: (a) hematological response at 1, 2, 3, 6 and 12 months and yearly thereafter; (b) relapse; (c) clonal evolution to PNH, clonal chromosomal population in bone marrow, myelodysplasia by morphology, or acute leukemia; (d) overall survival; (e) hematological response of relapse subjects that re-start treatment with cyclosporine and/or eltrombopag; (f) freedom from h-ATG.

Subjects will be followed up to 60 months so that long-term disease-free and overall survival can be estimated. Response and toxicity comparisons will be made with previous IST combined with eltrombopag protocol (12-H-0150)

9.3 SAMPLE SIZES AND STATISTICAL DESIGN

Cohort 1: Since safety and feasibility is defined in Section 9.1 to be the primary endpoint of this study, the treatment is defined to be "feasible" to a patient if he/she meets the safety and feasibility criteria specified in Section 9.1 at the end of study treatment period. Study treatment period is defined as the duration from the initiation of oral treatment to the start of standard of care treatment here at NIH. "Feasibility" is defined as tolerating treatment without the composite of TRSAE/misdiagnoses/noncompliance (defined in section 9.1). Our past experience with the standard 3 drug regimen (12-H-0150) suggests that the probability of feasibility for this patient population is approximately 90%. We hypothesize that the actual feasibility probability using this novel treatment paradigm may decrease to 75%, therefore a probability of 75% or less would warrant terminating this novel treatment approach. The hypotheses are equivalent to H_0: $p \leq 75\%$, in which feasibility is unacceptable, and H_1: $p \geq 90\%$, in which feasibility is acceptable. Our sample size is determined by testing this hypothesis at a significance level of 0.05 and 0.80 power. We intend to test this treatment approach using a small number of patients and terminate the study if early evidence suggests that the feasibility probability diminishes below the benchmark of 75%. We determine the sample size using the Two-Stage Minimax Design of Simon (1989), since it requires a smaller total number of subjects (n = 39) compared to the Two-Stage Optimal Design (n = 48). At the first stage, 22 subjects will be accrued, and the null hypothesis will be accepted if 17 or fewer subjects are determined to be feasible to the treatment within defined treatment period. Therefore, if 18 or more subjects are feasible to the treatment at the first stage, 17 additional subjects will be accrued. The null hypothesis of $p \leq 75\%$ will be accepted if the total number of patients feasible to the treatment are 33 or fewer.

After 39 patients are accrued on Cohort 1, we will start accruing patients on Extension Cohort. The 1-year HR clonal evolution rate of 8% from the 12-H-0150 protocol (Cohort 3 + Extension arm) is used as a benchmark for our population. The 1-year HR evolution rate in the 20-H-0033 study currently stands at 3.8%, with a 95% CI of [0, 10%]. Our objective is to achieve a more precise point estimate for the 1-year HR clonal evolution rate. If we increase the sample size to 80 patients total (Cohort 1 + Extension Cohort) and assume that the HR evolution rate will stay at 3.8%, our confidence width narrows to [0, 7.8%], the upper bound of which is below the 8% historical rate, in which case we can more confidently conclude a benefit in the rate of HR clonal evolution. With 39 subjects already accrued in this study, an additional 41 subjects will need to be recruited.

9.4 STATISTICAL METHODS

The planned analyses will be based on the "intention-to-treat" principle and include descriptive statistics on the proportions of feasible patients. The feasibility probability will be estimated using the sample proportion, and its inferences, including confidence intervals and hypotheses testing, will be evaluated using Binomial distributions. Survival analysis for time-to-death and time-to-response will include methods based on the Kaplan-Meier estimates and the Cox Proportional Hazard regression. If it is appropriate, we will consider additional analyses for the primary and secondary endpoints using the analysis of variance, multiple regression and logistic regression models. Graphical tools will be used to display the appropriate estimates (i.e.

estimated proportions and the Kaplan-Meier curves) and their corresponding 95% confidence intervals.

9.5 STOPPING RULES

In addition to the safety and feasibility criteria used in the primary endpoint (Section 9.1), we will also monitor a specified set of treatment related serious adverse events (TRSAEs) within the defined treatment period. This is the period of up to 8 weeks (or the allowed grace period in special circumstances defined in section 9.6) from initiation of oral therapy until evaluation at the clinical center. This safety monitoring is to ensure that the rate of TRSAEs does not substantially exceed an anticipated rate. The following TRSAEs will be monitored for early stopping of the study:

- Death considered to be probably or definitely related to the oral treatment during the study treatment period
- Any grade IV toxicities considered to be probably or definitely related to the treatment
- Death related to misdiagnosis and delay in appropriate treatment

The study will be monitored using the stopping rules as outlined below for early stopping if the number of subjects in the study who have developed one or more of the above specified TRSAEs is over a pre-specified threshold value. TRSAEs are those attributed as definitely or probably related to eltrombopag.

From our experience using this agent in other clinical settings, we anticipate the rate of developing at least one of the above specified TRSAEs for this patient population to be 20% or less. Our stopping rule is determined by a Bayesian approach based on Geller et al. publication[44]. The stopping boundary for the study is reached if the Bayesian posterior probability that the true probability of developing one or more of the above specified TRSAEs exceeds this benchmark rate of 20% is at least 90%. We take our prior distribution to be a beta distribution with the sum of the two beta parameters to be 5.5, i.e. the parameters of the beta prior distribution are 1.10 and 4.40. Since we have seen in the past that the first few subjects to be accrued are possibly sicker than the rest of the subjects in the sample, we will start safety monitoring the study when 3 or more subjects have developed specified TRSAEs. The following tables summarize the threshold numbers for stopping the study:

Number of Tx subjects enrolled	**Stop if the number of Tx subjects who develop any of the specified TRSAEs reaches:**
≤ 5	3
≤ 8	4
≤ 12	5
≤ 16	6
≤ 20	7
≤ 24	8

Number of Tx subjects enrolled	**Stop if the number of Tx subjects who develop any of the specified TRSAEs reaches:**
≤ 28	9
≤ 32	10
≤ 36	11
≤ 39	12

For a simulation study of the stopping rule, we generated a study with 39 independent Bernoulli trials, each had a probability p for having the above TRSAE and q=1-p for not having such TRSAE and compared the TRSAE outcomes with the above stopping boundary to determine whether the study was stopped. We repeated the simulation 100,000 times and computed the proportion of stopped studies (i.e. "number of stopped studies"/100,000), which were stopped using the above stopping rule. The following table summarizes the proportions of stopped studies under a number of scenarios for p:

Probability of Monitored" TRSAE= p	5%	10%	15%	20%	25%	40%
Proportion of stopped studies	0.15%	1.62%	7.26%	21.74%	44.48%	95.30%
Average number of Tx subjects	38.95	38.51	37.08	33.80	28.80	13.32
Average number of Tx with a specified TRSAE	1.95	3.85	5.56	6.76	7.20	5.33

The above results suggest that the above stopping rules have a low probability of stopping a study when the proportion of the corresponding specified TRSAE is below the benchmark value of 20%, and the probability of stopping a study is high when the true proportion of the above specified TRSAE exceeds this benchmark value. Based on these results, we believe that our Bayesian stopping rules have satisfactory statistical properties.

9.6 OFF TREATMENT AND OFF STUDY CRITERIA

All subjects will go off study after 5-year NIH landmark visit.

Subject choice: Subjects may be removed from study at their request at any time. The risks of withdrawing will be discussed, as will alternative treatment options. The risks of withdrawing from the study including high mortality rate with supportive care only (if that is chosen) will be discussed in detail with the patient. Those subjects who choose to

withdraw will be strongly encouraged to continue to have blood counts monitored until initiation of alternative SAA therapy or through the 6 months off study medication to assess for late occurring adverse events.

Principal investigator decision: Should any of the following events occur, study drug administration will be discontinued, and the subject will be followed until resolution of the event and labs will be monitored at least every week or as clinically indicated until he/she initiates alternative SAA therapy or through 6 months off study drug time point. Appropriate clinical experts such as ophthalmologist, etc. will be consulted as deemed necessary.

Adverse events related to:

- Death or any grade IV adverse event considered to be probably or definitely related to EPAG
- Life threatening acute hypersensitivity reactions
- Intolerance of eltrombopag not resolved by dose reduction
- Thrombosis/embolism (DVT, PE, stroke or TIA, myocardial infarction) other than line associated thrombosis
- Persistent hepatotoxicity as defined in section 6.4.2
- Infusion-related h-ATG reactions refractory to all appropriate supportive measures

Should any of the following events occur, study drug administration will be discontinued, and the subject will be taken off study.

Clonal evolution or relapse:

- Evidence of a poor risk clonal disorder (chromosome 7 abnormalities or complex cytogenetics) at any time while on study
- Evidence of myelodysplastic syndrome or AML according to WHO criteria at any time while on study
- Relapse criteria are met and treatment other than CsA and EPAG are started

Other

- Persistent non-compliance or lost to follow-up per PI discretion*
- Patients who require chemotherapeutic treatment or require drugs with hematological effects
- Initiation of additional immunosuppressive therapy other than steroids or cyclosporine (if beyond 6 months then mycophenolate mofetil can also be administered per PI discretion in patients who cannot tolerate cyclosporine and who require immunosuppression) or if beyond 2 years, enroll in other available clinical trials.

Pregnancy: This protocol does not enroll patients who are pregnant or women of child-bearing potential unless they are using contraception during the study treatment period (Section 4.3). Because aplastic anemia is a disease of young adults, if a woman becomes pregnant while participating in the study despite stringent contraception requirements and education by the study team regarding the risks of relapsed aplastic anemia with pregnancy upon study entry and at

every landmark visit, patient may remain on study. This limited inclusion of pregnant women on this study permits capture of the secondary endpoint (relapse of aplastic anemia) without exposing the subject/fetus/neonate to study drugs or invasive protocol procedures.

- If a patient becomes pregnant while on study drugs (CSA and/or EPAG), patient will discontinue the medications immediately but may remain on study
- If a patient becomes pregnant during follow-up period and not on any study treatments, patient may remain on study
- No investigational interventions or research procedures will be performed during pregnancy (if landmark visit is due during pregnancy, it will be delayed till after). All clinical decisions about pregnancy management will be made by the patient and the treating physician.
- If a patient relapses with aplastic anemia during pregnancy, no treatment will be provided on study; recommendations will be provided to the local treating physician for clinical management and if pursued any treatment other than supportive care (transfusions), patient will be taken off study
- Blood count monitoring with complete blood counts (CBCs) will be obtained with increased frequency than otherwise indicated, especially in later gestation, to monitor for AA relapse
- Fetus/neonate is not a research subject in this protocol
- No inducements, monetary, or otherwise, will be offered to terminate a pregnancy
- Individuals engaged in the research will have no part in any decisions as to the timing, method, or procedures used to terminate a pregnancy.
- Individuals engaged in the research will have no part in determining the viability of a neonate.
- At the conclusion of pregnancy, participant may remain on study, and resume protocol procedures for both safety and efficacy endpoints.

*****Please note that subjects not following up at the Clinical Center by 8 weeks or who fail to comply with the recommended oral treatment or clinical laboratory assessment may be taken off the study. However, per PI discretion, a "grace period" of additional 4 weeks may be granted if a patient is unable to present to the CC due to logistical reasons beyond their control such as inability to obtain visa, critical illness requiring hospitalizations etc., so long as they are responding to the oral treatment. During special circumstances such as COVID 19 outbreak, "grace period" may be extended up to 6 months per PI discretion.

Once off study, subjects will be referred back to the referring physician or consented to the NHLBI Standard of Care protocol (20H0099) for consideration for standard therapy or they may be evaluated for eligibility for another NIH protocol, depending on what is considered to be in the best interest of the subject.

10 SAFETY AND OTHER ASSESSMENTS

10.1 SAFETY MONITORING

Principal Investigator: Accrual, efficacy and safety data will be monitored by the Principal Investigator, Bhavisha Patel, M.D. Quality assurance and control monitoring will be consistent with the NHLBI Division of Intramural Research Clinical Research Quality Assurance and Quality Control Policy.

NHLBI DSMB*:* The NHLBI Data Safety and Monitoring Board will review the protocol at 6 to 12 month intervals. A progress report will be forwarded to the DSMB at these times and their recommendations will be expeditiously implemented. The DSMB may recommend early termination of the study for considerations of safety and efficacy.

Novartis: An annual progress report, any amendment to the protocol, and any change in the status of the protocol will be forwarded to Novartis.

10.2 ASSESSMENT OF SAFETY

Definitions

Adverse Event (AE): Any untoward medical occurrence in a human subject, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research (21 CFR 312.32 (a)).

Non-hematologic abnormal laboratory findings used to evaluate the safety of this protocol regimen will include any change from laboratory assessments done prior to first dose of study medication that result in a progression to a grade 3 or 4 laboratory toxicity or are characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

Serious Adverse Event (SAE): An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- results in death;
- is life-threatening (places the subject at immediate risk of death from the event as it occurred);
- results in in-patient hospitalization or prolongation of existing hospitalization;
- results in a persistent or significant incapacity;
- results in a congenital anomaly/birth defect; or

- based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

Suspected adverse reaction: Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

Unexpected adverse reaction: An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected", also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug but are not specifically mentioned as occurring with the particular drug under investigation.

Unanticipated problem (UP): Any incident, experience, or outcome that meets all of the following criteria:

- **Unexpected** in terms of nature, severity, or frequency in relation to
  - the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents; and
  - the characteristics of the subject population being studied; and
- **Related or possibly related** to participation in the research; and
- Places subjects or others at a **greater risk of harm** (including physical, psychological, economic, or social harm) than was previously known or recognized.

Unanticipated Problem that is not an Adverse Event: An unanticipated problem that does not fit the definition of an adverse event, but which may, in the opinion of the investigator, involves risk to the subject, affect others in the research study, or significantly impact the integrity of research data. For example, report occurrences of breaches of confidentiality, accidental destruction of study records, or unaccounted-for study drug.

Protocol Deviation (PD): Any change, divergence, or departure from the IRB approved research protocol.

Non-compliance: The failure to comply with applicable NIH HRPP policies, IRB requirements, or regulatory requirements for the protection of human research. Noncompliance may be further characterized as:

1. Serious non-compliance: Non-compliance that:

   a. Increases risks, or causes harm, to participants
   b. Decreases potential benefits to participants
   c. Compromises the integrity of the NIH HRPP
   d. Invalidates the study data

2. Continuing non-compliance: Non-compliance that is recurring. An example may be a pattern of non-compliance that suggests a likelihood that, absent an intervention, non-compliance will continue. Continuing noncompliance could also include a failure to respond to IRB requests to resolve previous allegations of non-compliance.

3. Minor (non-serious) non-compliance: Non-compliance that, is neither serious nor continuing. Safety assessments will consist of monitoring and recording AEs and SAEs; measurements of protocol-specified hematology, clinical chemistry, and other laboratory variables; measurement of protocol-specified vital signs; and other protocol-specified tests that are deemed critical to the safety evaluation of the study drug.

10.3 PROTOCOL DEVIATION (PD) REPORTING

It is anticipated that approximately 50% of the clinical monitoring laboratory testing performed during the first 6 months will not be performed within +/- 3 days of the required time points, with the exception of the month 3 and 6 landmark visits. If the clinical laboratory monitoring occurs outside the +/- 3 day window at a frequency greater than 50% in a single subject or greater than 50% cumulatively for all subjects it will be reported at time of continuing review. If the number of events per subject or per cumulative enrolled subjects exceeds 50%, this will be reported per Policy 801. Please note, subjects and providers are reminded of the importance of the timely completion of the clinical laboratory testing.

Interruptions in EPAG dosing that are clinically indicated per **section 5**, will only be recorded in the medical record. However, when the interruption is a consequence of a serious adverse event, the interruption will only be included in the SAE report.

In addition, CsA interruptions, such as delay in request for medication refills or medication errors by subjects, that are not clinically significant or impact the integrity of the research data, will only be recorded in the medical record.

10.4 ADVERSE EVENTS (AES) CHARACTERIZATION

The AEs will be attributed (unrelated, unlikely, possibly, probably or definitely related) to study medication and/or disease. The AEs will be graded by severity utilizing CTCAE version 5.0.

10.5 SERIOUS ADVERSE EVENTS (SAES) CHARACTERIZATION

Serious adverse events will be attributed as definitely (clearly related to the research), probably (likely related to the research), possibly (may be related to the research), unlikely (doubtfully related to the research) and unrelated (clear not related to the research).

TRSAEs are those attributed as definitely or probably related to treatment. As detailed in section 9.5 stopping rules, TRSAE that will be monitored and considered for early stopping the study according to statistically determined criteria include Death and any grade IV toxicity considered to be probably or definitely related to study medication.

Note that hospitalizations for the following reasons will not be captured as serious adverse events, as these hospitalizations are not a result of an adverse event:

- Routine treatment or monitoring, not associated with any deterioration in condition
- Hospitalizations for administrative issues (e.g., to receive a transfusion after hours) or movement to the ICU for routine monitoring per administrative requirements or nosocomial isolation
- Elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since signing the informed consent
- Social reasons and respite care in the absence of any deterioration in the patient's general condition

10.6 EVENT COLLECTION

10.6.1 Duration of Adverse Event Collection

The collection and recording of AEs will begin on the first day of initiation of the study drug and will continue for 30 days after the 6 month landmark visit or for 30 days after treatment is discontinued if before the 6 month landmark visit, after which the events will be captured in the medical record but not abstracted or recorded in the database. AEs that are abstracted in the database will be followed until satisfactory resolution.

10.6.2 Duration of Serious Adverse Event Collection

The collection of SAEs will begin on the first day of initiation of the study drug and will continue as long as the subject is on study. Starting 30 days after the 6 month landmark visit or 30 days after treatment is discontinued (if before the 6 month landmark visit), SAEs will only be captured in the research database when deemed possibly, probably or definitely related to the study treatment.

10.7 REPORTING EVENTS

All events listed in section10.2 will be reported to Bhavisha Patel, M.D., Principal Investigator of this study:

Bhavisha Patel, M.D.

Bldg. 10, Room CRC 3-5132

Phone: (301) 402-3477

E-mail: bhavisha.patel@nih.gov

10.7.1 Expedited Reporting to the NIH Intramural IRB

Events requiring expedited reporting will be submitted to the IRB per HRPP Policy 801 "Reporting Research Events".

10.7.2 Reports to the NIH Intramural IRB at the time of Continuing Review (CR)

The PI or designee will refer to HRPP Policy 801 "Reporting Research Events" to determine IRB reporting requirements.

10.7.3 Reports to the NHLBI Clinical Director (CD)

The PI or designee will refer to NHLBI DIR guidelines to determine CD reporting requirements and timelines.

10.7.4 Reporting Serious Adverse Events to a collaborator under Cooperative Research and Development Agreement (CRADA)

Novartis:

All unexpected and possibly, probably or definitely related SAEs occurring during the study or within 30 days of the last administration of eltrombopag will be reported to Novartis within 24 hours of the research team learning of the event. A copy of the SAE report (NHLBI SAE report form) will be forwarded as soon as possible, but no later than seven (7) days in the case of death or life-threatening serious adverse events or within fifteen (15) days after the occurrence of all other forms of serious adverse events. If the SAE is unexpected and determined possibly, probably or definitely related to the study drug the SAE report (in the appropriate format, e.g., NHLBI SAE report form, narrative) will be forwarded to Novartis within 24 hours of learning of event. The investigator must assess and record the relationship of each SAE to each specific study treatment (if there is more than one study treatment), complete the SAE Report Form in English, and send the completed, signed form along with the Novartis provided fax cover sheet to the Novartis Oncology Drug Safety and Epidemiology (DS&E) department by e-mail (clinicalsafetyop.phuseh@novartis.com) or by fax (fax: 877-778-9739) within 24 hours.

Any additional information for the SAE including complications, progression of the initial SAE, and recurrent episodes must be reported as follow-up to the original episode within 24 hours of the investigator receiving the follow-up information. An SAE occurring at a different time interval or otherwise considered completely unrelated to a previously reported one should be reported separately as a new event. Any SAEs experienced after the 30 day safety evaluation follow-up period (or 5 half-lives, if half-life is established, whichever is longer) should only be reported to Novartis if the investigator suspects a causal relationship to the study treatment.

Follow-up information is submitted in the same way as the original SAE Report. Each re-occurrence, complication, or progression of the original event should be reported as a follow-up to that event regardless of when it occurs. The follow-up information should describe whether the event has resolved or continues, if and how it was treated, whether the blind was broken or not, and whether the patient continued or withdrew from study participation.

If the SAE is not previously documented in the Investigator's Brochure or Package Insert (new occurrence) and is thought to be related to the Novartis study treatment, an oncology Novartis Drug Safety and Epidemiology (DS&E) department associate may urgently require further information from the investigator for Health Authority reporting. Novartis may need to issue an Investigator Notification (IN), to inform all investigators involved in any study with the same drug that this SAE has been reported. Suspected Unexpected Serious Adverse Reactions (SUSARs) will be collected and reported to the competent authorities and relevant ethics committees in accordance with Directive 2001/20/EC or as per national regulatory requirements in participating countries.

10.7.5 Reporting of Pregnancy

Subjects who become pregnant during the active treatment portion of the study (CsA and eltrombopag) should discontinue the study drugs immediately. See Section 4.3 and 9.6 for handling of pregnancy. The investigator, or his/her designee, will collect pregnancy information

on any subject who becomes pregnant while receiving study treatment or during the post-treatment follow-up period.

The investigator, or his/her designee, will submit pregnancy information to the NHLBI Clinical Director and Novartis within two weeks of learning of a subject's pregnancy.

Pregnancy will be reported by the investigator to the Novartis Oncology Drug Safety and Epidemiology Department (DS&E) by fax (fax: 877-778-9739) or via email (**clinicalsafetyop.phuseh@novartis.com)**. Information on the status of the mother and child will be forwarded to Novartis. Generally, follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any premature termination of the pregnancy will be reported.

The pregnancy will be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications. While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be recorded and reported to Novartis as an AE or SAE. A spontaneous abortion is always considered to be an SAE and will be reported to Novartis. Furthermore, any SAE occurring as a result of a post-study pregnancy and is considered reasonably related to the investigational product by the investigator, will be reported to Novartis. While the investigator is not obligated to actively seek this information in former study participants, he/she may learn of an SAE through spontaneous reporting. Pregnancy outcomes for the female partners of any males who took study treatment on this study will also be collected.

Further advice on the length of post-natal follow up may be sought from the Novartis Pediatric Advisory Group and should be driven by the type of congenital abnormality expected.

10.8 PROTOCOL MONITORING

As per ICH-GCP 5.18 and FDA 21 CFR 312.5 clinical protocols are required to be adequately monitored by the study sponsor. The monitoring of this study will be conducted by Clinical Research Associates (CRAs)/Monitors working under an agreement with NHLBI to monitor aspects of the study in accordance with the appropriate regulations and the approved protocol. The objectives of a monitoring visit will be: 1) to verify the existence of signed informed consent form (ICF) and documentation of the ICF process for each monitored subject; 2) to verify the prompt and accurate recording of all monitored data points, and prompt reporting of all SAEs; 3) to compare abstracted information with individual subjects' records and source documents (subject's charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original subject information); and 4) to help ensure investigators are in compliance with the protocol. The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements (Office for Human Research Protections-OHRP), FDA and applicable guidelines (ICH-GCP) are being followed. During the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

The investigator (and/or designee) will make study documents (e.g., consent forms and pertinent hospital or clinical records readily available for inspection by the local IRB, the FDA, the site monitors, and the NHLBI staff for confirmation of the study data.

11 BIOSPECIMEN AND DATA MANAGEMENT PLAN

Biospecimen Management: Specimens and their derivatives (e.g., genomic material, cell lines) will be coded and stored in conformity with DIR Policy (e.g., BSI). Coded biospecimens may be sent to collaborators outside of the NIH in accordance with applicable NIH and DIR Policy for sharing research resources, including an executed material transfer agreement. Biospecimens with subject personal identifiers may be sent to associate investigators and collaborators outside of the NIH only after approvals of all applicable IRBs, an executed reliance agreement with NIH's IRB, or an extension of the NIH's FWA through an Individual Investigator Agreement.

Storage: All samples will be stored in the laboratory of Dr. Young. Collected samples will be de-identified prior to storage in the laboratory of the principal investigator following current NHLBI DIR BSI Policy. Efforts to ensure protection of patient information include:

- Each sample is assigned a unique number
- Vials holding patient samples are labeled with the sequential laboratory accession ID number that does not contain any personal identifier information
- An electronic database is used to store patient information related to the coded samples
- The laboratory is located in a controlled access building and laboratory doors are kept locked. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.
- Hard copy records or electronic copies of documents containing patient information are kept in the locked laboratory or other controlled access locations.

Biospecimen Tracking and Sharing: Samples will be ordered and tracked through the CRIS Research Screens. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical record.

Coded biospecimens (NHLBI investigators hold the key that identifies research subjects) to be shared outside of NIH for future research use requires an executed transfer agreement and may require IRB approval if results will be returned and re-identified.

Unlinked biospecimens (no key to identify research subjects exists) to be shared outside of NIH for future research use requires an executed transfer agreement. Refusal of a research subject participant to allow for future use of identifiable biospecimens--other than required in the protocol or for appropriate regulatory purposes, e.g., by the FDA--will be honored.

Future use of biospecimens: Following analyses of biospecimens for primary research purposes, remaining samples suitable for future research will be stored in manner that conforms with DIR policy (such as BSI) or in a publicly accessible research biospecimen repository following IRB approval, as applicable. Biospecimens may be destroyed only when permitted by the clinical director and the IRB.

Data Management: The principal investigator, associate investigators, research nurses and/or a data manager will assist with the data management efforts. Data will be abstracted from Clinical Center progress notes as well as intake forms and the case report forms. Laboratory data from NIH will be reviewed using CRIS. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts to ensure that data is verifiable and evaluable. Data will be abstracted from Clinical Center progress notes as well as from progress notes forwarded from the subjects' home physician.

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system and ensuring data accuracy, consistency and timeliness. Laboratory values from referring home physicians will be entered into the system. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts to ensure that data is verifiable and evaluable. Data will not be sent outside of the NIH without institutional approval.

Research data will be prospectively collected by authorized Investigator personnel and entered into an NHLBI approved Database. The database is a web-based, 21 CFR 11 compliant database which will consist of the study specific set of electronic CRFs (e-CRFs) used for capturing, managing and reporting clinical research data.

The database will maintain complete data records on each research subject.

- White blood cell count
- Absolute lymphocyte count (percentage if absolute not available)
- Absolute neutrophil count (percentage if absolute not available)
- Absolute reticulocyte count, if available (percentage if absolute not available, then also need the red blood cell count)
- Hemoglobin
- Platelets

Subjective and objective patient experiences during the duration of the study will be documented in the patient medical record notes. These protocol notes will serve as the primary source material from which data will be collected in the Database. Any pertinent supplementary information obtained from outside laboratories, outside hospitals, radiology reports, laboratory reports, or other patient records will be used as additional source for data collection.

We will maintain the confidentiality of identifiable private information collected in this Clinical Trial and protect the privacy of the individual human subjects. Primary data containing individually identifiable information obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH information security standards. Neither individual personal identifiers nor the key linking coded data to individuals will be released without prior IRB approval and an executed CDA or MTA. Identifiable data will not be sent outside NIH without prior IRB approval or appropriate conditions for disclosure outlined in the executed CDA or MTA. Study data will be housed in the Hematology Branch P Drive, a secure limited access drive.

Novartis will receive quarterly accrual and toxicity information as detailed in the CRADA. In order to maintain subject confidentiality, all communications relating to the study will identify participants by assigned subject study numbers. No personally identifiable information will be sent to Novartis. In accordance with local and federal regulations, the Investigator will allow Novartis personnel or their designee, access to all pertinent medical records in order to verify the data gathered and to audit the data collection process.

Data sharing and future use of data: Research data may be shared with qualified non-collaborator recipients following publication of the primary research results after removal of PII. Refusal of a research subject participant to permit future use of data--other than required in the protocol or by the FDA--will be honored. Limitations in data sharing and future use of data due

to contractual obligations (e.g., CRADAs) or intellectual property proceedings (such as patent filings) will be honored.

End of study procedures: Data will be stored in locked cabinets and in a password protected database until it is no longer of scientific value. At the completion of the protocol (termination), samples and data will be maintained in a repository for future research.

Loss or destruction of data: Should we become aware that a major breech in our plan to protect patient confidentiality and trial data has occurred, the IRB will be notified.

Privacy and Confidentiality: All efforts, within reason, will be made to keep subjects' private identifiable information (PII) private. Using or sharing ("disclosure") such data must follow federal privacy rules. Under certain circumstances, the United States Office of Human Research Protections (OHRP), The US Food and Drug Administration (FDA), and the IRB, will be able to inspect and copy confidential study-related records which identify participants by name. Therefore, absolute confidentiality cannot be guaranteed.

12 HUMAN SUBJECT PROTECTION

12.1 RATIONALE FOR SUBJECT SELECTION

The study will be open to all subjects who satisfy the inclusion criteria and provide an informed consent to the protocol. No subjects will be excluded from participation based on gender, race or ethnicity. We do not anticipate enrollment of NIH staff. This is a rare and debilitating disease. To our knowledge, currently, there are no NIH staff that would qualify for this study.

This study will be open to all patients who fit the inclusion criteria and provide informed consent to protocol participation. Epidemiologic studies suggest that the gender will be approximately evenly split between male and females, and that 90% of the patients will be Caucasian. However, previous experience at our institution suggests that distribution will be:

- By gender: 48% males and 52% females;
- By race/ethnicity: approximately 58% White, 20% Black, 10% Asian and 17% Hispanic;
- By age: will range between 3 and 82 (median age of 30) and roughly 20% of patients will be under the age of 18.

For subjects of Asian ethnicity: Plasma eltrombopag area under the curve was approximately 70% higher in East and Southeast Asian (ethnicity self-reported) subjects as compared to non-Asian subjects who were predominantly Caucasian. Therefore, subjects of Asian heritage will be included but they will be initiated at a lower dose and monitored closely as described in the treatment plan.

For subjects with renal impairment: The pharmacokinetics of eltrombopag has been studied in adult patients with renal impairment. Following administration of a single 50 mg dose, there was a trend for reduced plasma eltrombopag exposure in patients with renal impairment, but there was substantial variability and significant overlap in exposures between patients with renal impairment and healthy volunteers. Therefore, patients with impaired renal function will be included and given the protocol-defined dosages, but participation will be monitored closely.

For subjects with hepatic impairment: Pharmacokinetics of eltrombopag has been studied in adult patients with hepatic impairment. Following the administration of a single 50 mg dose, the AUC0-∞ of eltrombopag was increased by 41% in subjects with mild hepatic impairment and by

80% to 90% in subjects with moderate or severe hepatic impairment compared with healthy volunteers. Therefore, patients with minimally impaired hepatic function will be included but participation will be monitored closely. Patients with baseline moderate to severe hepatic impairment will be excluded from the study.

Recruitment efforts:

A Strategic Recruitment Plan (SRP) will be developed with the NHLBI Patient Recruitment Office (PRO). The recruitment strategies to be used for this study may include:

- Social media postings which may include NHLBI and Clinical Center accounts for Facebook and Twitter
- Listings on official NIH websites to include:
    1. Clinicaltrials.gov website
    2. Clinical Center Recruitment website with dedicated study page mirroring the patient recruitment flyer. Page links to CT.gov and/or Search the Studies.
    3. Clinical Center Research Studies ("Search the Studies") website
- The AAMDS website and/or social media accounts (using IRB approved language, flyer, and/or social media language with photos.)
- Use of Clinical Center Office of Patient Recruitment Services (OPR) including OPR Listservs *(Email list of those interested in receiving study recruitment updates.) (Links to one of the above mentioned websites or pages)*. Includes the OPR Protocols and OPR Healthy Volunteers listservs and 3 NIH listservs (NIH Post back, NIH Clinical Fellows & NIH Study Volunteers),
- ResearchMatch, NIH Newsletters
- Hematologists and Oncologists in the US will be informed about the protocol via email. An informational letter to physicians will be available for electronic (and hard copy as appropriate) distribution to local and national clinical contacts.

If recruitment goals are not being met, additional strategies will be planned and implemented by the PRO.

Reimbursement for protocol travel, food, and lodging will be consistent with NIH guidelines. In determining reimbursement, the following factors are considered applicable to this protocol: the patients are diagnosed with a rare disease; the patient population is sick; the protocol offers the potential for direct benefit; the protocol regimen is demanding; and in order to complete accrual in a reasonable timeframe a geographically dispersed participant population is required.

Payment for participation $0. The study participants will not be reimbursed for their time and inconvenience.

Competition between Branch Protocols: There are no competing Branch protocols for this patient population.

12.2 PARTICIPATION OF CHILDREN

In principle, age is not a consideration. But in practice, we are limiting the protocol to subjects who are age 3 years and older because our clinic does not have the expertise to care for infants. In addition, per Clinical Center guidelines, we are limiting participation to children who weigh >12kg.

12.3 RISKS AND DISCOMFORTS

12.3.1 Related to Horse ATG (ATGAM)

Anaphylaxis (less than 1% of patients): Rarely, patients may develop potentially fatal anaphylaxis. Production by the patient of antibodies to horse proteins leads to the formation of immune complexes and the clinical development of serum sickness, characterized by fever, a characteristic rash, arthralgia, myalgia and non-specific gastrointestinal and neurologic symptoms. Onset is typically at day ten to eleven, and the course is self-limited; symptoms may be improved by corticosteroids. Transient reduction in peripheral granulocyte and platelet counts and in the hemoglobin may occur during the period of administration of ATG and may lead to a temporary increase in transfusion requirements.

Severe lung injury (less than 1% of patients): Several cases of a severe lung injury related to Atgam treatment have been reported. Although this side effect appears extremely rare, it is serious and can be fatal. There is no information about the mechanism or specific treatment for this condition. A few patients recovered after intensive medical support including use of a breathing machine.

Cardiac Failure and Pulmonary Edema (less than 5% of patients): h-ATG is associated with pulmonary edema or congestive heart failure.

Potential Serious Adverse Effects:

Potential side effects related to h-ATG (ATGAM) include:

Very common side effects (occurring in 10% or more of patients): fever (51%), chills (16%), thrombocytopenia (30%), leukopenia (14%), skin rash (27%)

Common side effects (occurring in 5 to 10% of patients): serum sickness like symptoms, dyspnea/apnea, arthralgia, headache, chest, back or flank pain, diarrhea and nausea and/or vomiting

Events reported with frequency of less than 5% of patients in a pre-marketing clinical trial in the treatment of aplastic anemia include: diaphoresis, joint stiffness, peri-orbital edema, aches, edema, muscle ache, vomiting, agitation, lethargy, listlessness, light-headedness, seizures, diarrhea, bradycardia, myocarditis, cardiac irregularity, hepatosplenomegaly, post viral encephalopathy, hypotension, congestive heart failure, hypertension, burning soles/palms, foot sole pain, lymphadenopathy, post cervical lymphadenopathy, tender lymph nodes, pleural effusion, respiratory distress, and proteinuria.

Related to pregnancy and/or nursing mothers: h-ATG has not been evaluated in either pregnant or lactating women therefore administration of h-ATG to pregnant women is not recommended and should be considered only under exceptional circumstances.

Post marketing Experience:

During approximately 5 years of post-approval marketing experience, the frequency of adverse reactions in voluntarily reported cases is as follows: fever 51%; chills 16%; thrombocytopenia

30%; leukopenia 14%; rashes 27%; systemic infection 13%. Events reported in 5% to 10% of reported cases include abnormal renal function tests; serum sickness-like symptoms; dyspnea/apnea; arthralgia; chest, back, or flank pain; diarrhea and nausea and/or vomiting. Events reported with a frequency of less than 5% include: hypertension, Herpes Simplex infection, pain, swelling or redness at infusion site, eosinophilia, headache, myalgias, or leg pains, hypotension, anaphylaxis, tachycardia, edema, localized infection, malaise, seizures, GI bleeding or perforation, deep vein thrombosis, sore mouth/throat, hyperglycemia, acute renal failure, abnormal liver function tests, confusion or disorientation, cough, neutropenia or granulocytopenia, anemia, thrombophlebitis, dizziness, epigastric or stomach pain, lymphadenopathy, pulmonary edema or congestive heart failure, abdominal pain, nosebleed, vasculitis, aplasia or pancytopenia, abnormal involuntary movement or tremor, rigidity, sweating, laryngospasm/edema, hemolysis or hemolytic anemia, viral hepatitis, faintness, enlarged or ruptured kidney, paresthesia, and renal artery thrombosis.

12.3.2 Related to CsA

Potential Serious Side Effects Include:

Infection related: Because of low white blood cell counts, patients with aplastic anemia are susceptible to infections. By further blocking the immune system, CsA further increases this risk.
Cancer related: When used at high doses in transplant patients, CsA may be associated with an increased risk of cancer, especially lymphoma (4 of every 10,000 patients who receive the medication). Transplant patients receive higher doses than you will be given and are treated for longer periods than the duration of this study. However, because of the way that CsA acts on the body, there is a chance that it may cause effects that may not occur until years after the medicine is used.
Blindness: In very rare instances (less than .01%), CsA has been reported to cause blindness

Potential side effects:

Although it is metabolized primarily in the liver, CsA major toxicity is renal. CsA causes a decrease in creatinine clearance, which almost always returns to normal range on cessation of the drug or lowering of the dose. Rare development of a hemolytic-uremic syndrome has been reported in patients with CsA after allogeneic bone marrow transplant. In our patients with SAA, frequent creatinine measurements have allowed prompt adjustment of dose and serious renal complications are infrequent.

Evidence of hepatotoxicity is common, usually as transient increases in bilirubin and transaminases. These levels often normalize with continued administration of the drug; reduction of the dose is uniformly associated with a return to normal levels.

Additional complications include hypertrichosis, gingival hypertrophy (possibly related to pre-existing poor dental hygiene), hyperesthesia, hirsutism, tremors, headaches, nausea and nonspecific gastrointestinal complaints. Hypertension may occur and be high enough to require treatment.

Neurologic complications include insomnia, dizziness, anxiety, confusion, and vertigo. We have observed seizures in patients receiving CsA, when drug levels were within the

therapeutic range. Posterior Reversible Encephalopathy Syndrome (PRES) is an increasingly recognized neurologic disorder seen in 1% of patients on cyclosporine following solid organ transplantation which manifest with acute to subacute hypertension and/or seizures. In the event of hypertension, subjects will be prescribed 1 or more medications to control blood pressure in an effort to decrease the risk of this complication.

Hypomagnesemia and hyperkalemia may occur but are asymptomatic. Increases in uric acid may occur and attacks of gout have been rarely reported. Cyclosporine therapy may be associated with a modest increase of serum triglycerides or cholesterol.

Less frequent adverse events include:

Autonomic Nervous System: dry mouth, increased sweating
Systemic: allergy, asthenia, hot flushes, malaise, weight decrease, weight increase
Cardiovascular: abnormal heart sounds, cardiac failure, myocardial infarction, peripheral ischemia
Central and Peripheral Nervous System: hypoesthesia, neuropathy, vertigo
Endocrine: goiter
Gastrointestinal: constipation, dysphagia, enanthema, eructation, esophagitis, gastric ulcer, gastritis, gastroenteritis, gingival bleeding, glossitis, peptic ulcer, salivary gland enlargement, tongue disorder, tooth disorder
Infection: abscess, bacterial infection, cellulitis, folliculitis, fungal infection, herpes simplex, herpes zoster, renal abscess, moniliasis, tonsillitis, viral infection
Hematologic: anemia, epistaxis, leukopenia, lymphadenopathy
Liver and Biliary System: bilirubinemia
Metabolic and Nutritional: diabetes mellitus, hyperkalemia, hyperuricemia, hypoglycemia
Musculoskeletal System: arthralgia, bone fracture, bursitis, joint dislocation, myalgia, stiffness, synovial cyst, tendon disorder
Neoplasms: breast fibroadenosis, carcinoma
Psychiatric: anxiety, confusion, decreased libido, emotional lability, impaired concentration, increased libido, nervousness, paranoia, somnolence
Reproductive (Female): breast pain, uterine hemorrhage
Respiratory System: bronchospasm
Skin and Appendages: abnormal pigmentation, angioedema, dermatitis, dry skin, eczema, nail disorder, pruritus, skin disorder, urticaria
Special Senses: abnormal vision, cataract, conjunctivitis, deafness, eye pain, taste perversion, tinnitus, vestibular disorder, blindness
Urinary System: abnormal urine, hematuria, increased BUN, micturition urgency, nocturia, polyuria, pyelonephritis, urinary incontinence

<u>Prolonged low dose administration of CsA:</u>

Patients who undergo solid organ transplantation, such as kidney transplantation, are given cyclosporine for life at a high dose (usually 5-10 mg/kg/day) whereas we are proposing to continue at low dose, 2mg/kg/day. Cyclosporine is considered a weak immunosuppressant and few infectious complications are observed. There is ample data on the use of cyclosporine from long-term studies where cyclosporine is used for solid organ transplantation. Nephrotoxicity is the main toxicity, usually reversible, but is typically only observed when troughs over 200ng/mL are

targeted. Nephrotoxicity is rarely observed with low-dose cyclosporine and monitoring of levels is unnecessary. The risk of relapse of severe aplastic anemia; however, can be fatal, and this risk far exceeds the risks long term cyclosporine poses as is evidenced by our experience and data from 12-H-0150.

12.3.3 Related to Promacta (eltrombopag).

Potential Serious Adverse Effects:

Warnings and Precautions

WARNING: RISK FOR HEPATIC DECOMPENSATION IN PATIENTS WITH CHRONIC HEPATITIS C RISK OF HEPATOTOXICITY See full prescribing information for complete boxed warning. In patients with chronic hepatitis C, PROMACTA in combination with interferon and ribavirin may increase the risk of hepatic decompensation. PROMACTA may increase the risk of severe and potentially life-threatening hepatotoxicity. Monitor hepatic function and discontinue dosing as recommended.

Hepatic Decompensation in Patients with Chronic Hepatitis C

In patients with chronic hepatitis C, PROMACTA in combination with interferon and ribavirin may increase the risk of hepatic decompensation. In two controlled clinical trials in patients with chronic hepatitis C and thrombocytopenia, ascites and encephalopathy occurred more frequently on the arm receiving treatment with PROMACTA plus antivirals (7%) than the placebo plus antivirals arm (4%). Patients with low albumin levels (less than 3.5 g/dL) or Model for End-Stage Liver Disease (MELD) score greater than or equal to 10 at baseline had a greater risk for hepatic decompensation on the arm receiving treatment with PROMACTA plus antivirals. Discontinue PROMACTA if antiviral therapy is discontinued.

Hepatotoxicity

PROMACTA may increase the risk of severe and potentially life-threatening hepatotoxicity. Measure serum ALT, AST, and bilirubin prior to initiation of PROMACTA, every 2 weeks during the dose adjustment phase, and monthly following establishment of a stable dose. PROMACTA inhibits UDP- glucuronosyltransferase (UGT)1A1 and organic anion-transporting polypeptide (OATP)1B1, which may lead to indirect hyperbilirubinemia. If bilirubin is elevated, perform fractionation. Evaluate abnormal serum liver tests with repeat testing within 3 to 5 days. If the abnormalities are confirmed, monitor serum liver tests weekly until resolved or stabilized. Discontinue PROMACTA if ALT levels increase to greater than or equal to 3 x ULN in patients with normal liver function or greater than or equal to 3 x baseline (or greater than 5 x ULN, whichever is the lower) in patients with pre-treatment elevations in transaminases and are:

- progressively increasing, or
- persistent for greater than or equal to 4 weeks, or
- accompanied by increased direct bilirubin, or
- accompanied by clinical symptoms of liver injury or evidence for hepatic

decompensation.

If the potential benefit for reinitiating treatment with PROMACTA is considered to outweigh the risk for hepatotoxicity, then consider cautiously reintroducing PROMACTA and measure serum liver tests weekly during the dose adjustment phase. Hepatotoxicity may reoccur if PROMACTA is reinitiated. If liver test abnormalities persist, worsen, or recur, then permanently discontinue PROMACTA.

Isolated cases of severe liver injury were identified in clinical trials. The elevation of liver laboratory values occurred approximately three months after initiation of PROMACTA. In all cases, the event resolved following PROMACTA discontinuation.

Thrombotic/Thromboembolic Complications

Thrombotic/thromboembolic complications may result from increases in platelet counts with PROMACTA. Reported thrombotic/thromboembolic complications included both venous and arterial events and were observed at low and at normal platelet counts.

Consider the potential for an increased risk of thromboembolism when administering PROMACTA to patients with known risk factors for thromboembolism (e.g., Factor V Leiden, ATIII deficiency, antiphospholipid syndrome, chronic liver disease). To minimize the risk for thrombotic/thromboembolic complications, do not use PROMACTA in an attempt to normalize platelet counts. Follow the dose adjustment guidelines to achieve and maintain target platelet counts.

In two controlled clinical trials in patients with chronic hepatitis C and thrombocytopenia, 3% (31/955) treated with PROMACTA experienced a thrombotic event compared with 1% (5/484) on placebo. The majority of events were of the portal venous system (1% in patients treated with PROMACTA versus less than 1% for placebo).

In a controlled trial in patients with chronic liver disease and thrombocytopenia not related to ITP undergoing elective invasive procedures (N = 292), the risk of thrombotic events was increased in patients treated with 75 mg of PROMACTA once daily. Seven thrombotic complications (six patients) were reported in the group that received PROMACTA and three thrombotic complications were reported in the placebo group (two patients). All of the thrombotic complications reported in the group that received PROMACTA were portal vein thrombosis (PVT). Symptoms of PVT included abdominal pain, nausea, vomiting, and diarrhea. Five of the six patients in the group that received PROMACTA experienced a thrombotic complication within 30 days of completing treatment with PROMACTA and at a platelet count above 200 x 10^9/L. The risk of portal venous thrombosis was increased in thrombocytopenic patients with chronic liver disease treated with 75 mg of PROMACTA once daily for 2 weeks in preparation for invasive procedures.

Cataracts

In the three controlled clinical trials in adults with chronic ITP, cataracts developed or worsened in 15 (7%) patients who received 50 mg of PROMACTA daily and 8 (7%) placebo-group patients. In the extension trial, cataracts developed or worsened in 11% of patients who underwent ocular examination prior to therapy with PROMACTA. In the two controlled clinical trials in patients with chronic hepatitis C and thrombocytopenia, cataracts developed or worsened in 8% of patients treated with PROMACTA and 5% of patients treated with placebo.

Cataracts were observed in toxicology studies of eltrombopag in rodents. Perform a baseline ocular examination prior to administration of PROMACTA and, during therapy with PROMACTA, regularly monitor patients for signs and symptoms of cataracts.

Clinical Experience:

For full information on clinical experience with eltrombopag in for the treatment of all approved indications, see PACKAGE INSERT.

Severe Aplastic Anemia: In the single-arm, open-label trial, 43 patients with severe aplastic anemia received PROMACTA. Eleven patients (26%) were treated for greater than 6 months and 7 patients (16%) were treated for greater than 1 year. The most common adverse reactions (greater than or equal to 20%) were nausea, fatigue, cough, diarrhea, and headache.

Adverse Reactions (≥10%) from One Open-label Trial in Adults with Severe Aplastic Anemia

Adverse Reaction	**PROMACTA (n = 43) (%)**
Nausea	33
Fatigue	28
Cough	23
Diarrhea	21
Headache	21
Pain in extremity	19
Dyspnea	14
Pyrexia	14
Dizziness	14
Oropharyngeal pain	14
Febrile neutropenia	14
Abdominal pain	12
Ecchymosis	12
Muscle spasms	12
Transaminases increased	12
Arthralgia	12
Rhinorrhea	12

Rash was reported in 7% of patients.

In this trial, patients had bone marrow aspirates evaluated for cytogenetic abnormalities. Eight patients had a new cytogenetic abnormality reported on therapy, including 5 patients who had complex changes in chromosome 7.

Post marketing Experience

Thrombotic microangiopathy with acute renal failure have been identified during post approval use of PROMACTA. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate the frequency or establish a causal relationship to drug exposure.

USE IN SPECIFIC POPULATIONS

Pregnancy

Pregnancy Category C

There are no adequate and well-controlled studies of eltrombopag use in pregnancy. In animal reproduction and developmental toxicity studies, there was evidence of embryolethality and reduced fetal weights at maternally toxic doses. PROMACTA should be used in pregnancy only if the potential benefit to the mother justifies the potential risk to the fetus.

In an early embryonic development study, female rats received oral eltrombopag at doses of 10, 20, or 60 mg/kg/day (0.8, 2, and 6 times, respectively, the human clinical exposure based on AUC in patients with ITP at 75 mg/day and 0.3, 1, and 3 times, respectively, the human clinical exposure based on AUC in patients with chronic hepatitis C at 100 mg/day). Increased pre- and post-implantation loss and reduced fetal weight were observed at the highest dose which also caused maternal toxicity.

Eltrombopag was administered orally to pregnant rats at 10, 20, or 60 mg/kg/day (0.8, 2, and 6 times, respectively, the human clinical exposure based on AUC in patients with ITP at 75 mg/day and 0.3, 1, and 3 times, respectively, the human clinical exposure based on AUC in patients with chronic hepatitis C at 100 mg/day). Decreased fetal weights (6% to 7%) and a slight increase in the presence of cervical ribs were observed at the highest dose which also caused maternal toxicity. However, no evidence of major structural malformations was observed.

Pregnant rabbits were treated with oral eltrombopag doses of 30, 80, or 150 mg/kg/day (0.04, 0.3, and 0.5 times, respectively, the human clinical exposure based on AUC in patients with ITP at 75 mg/day and 0.02, 0.1, and 0.3 times, respectively, the human clinical exposure based on AUC in patients with chronic hepatitis C at 100 mg/day). No evidence of fetotoxicity, embryolethality, or teratogenicity was observed.

In a pre- and post-natal developmental toxicity study in pregnant rats (F0), no adverse effects on maternal reproductive function or on the development of the offspring (F1) were observed at doses up to 20 mg/kg/day (2 times the human clinical exposure based on AUC in patients with ITP at 75 mg/day and similar to the human clinical exposure based on AUC in patients with chronic hepatitis C at 100 mg/day). Eltrombopag was detected in the plasma of offspring (F1). The plasma concentrations in pups increased with dose following administration of drug to the F0 dams.

Nursing Mothers

It is not known whether eltrombopag is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from PROMACTA, a decision should be made whether to discontinue nursing or to discontinue PROMACTA taking into account the importance of PROMACTA to the mother.

Pediatric Use

The safety and efficacy of PROMACTA in pediatric patients 1 year and older with chronic ITP were evaluated in two double-blind, placebo-controlled trials. The pharmacokinetics of eltrombopag have been evaluated in 168 pediatric patients 1 year and older with ITP dosed once daily. The safety and efficacy of PROMACTA in pediatric patients younger than 1 year with ITP have not yet been established.

The safety and efficacy of PROMACTA in pediatric patients with thrombocytopenia associated with chronic hepatitis C and severe aplastic anemia have not been established.

Adverse reactions

Severe cutaneous reaction

There is a risk that subject may develop a severe cutaneous reaction that may require hospitalization and discontinuation of EPAG. As of November 2018, there have been four occurrences of this event in subjects enrolled in 12-H-0150, our previous trial.

Adverse Drug Reactions (ADRs) selected based upon the weight of all available information, including data from the Novartis safety database (Argus), and data from all completed global clinical trials (phase I-III) for which the Clinical Study Report is final or there is an appropriate final DMC report, up to 28 February 2017 are presented in table below. Please also refer to the Investigator Brochure (IB).

Adverse reactions are listed below for each indication by MedDRA body system organ class and by frequency. Frequency category for each adverse drug reaction is based on the following convention (CIOMS III). The frequency categories used are:

Very common: ≥1 in 10 (≥10%)

Common: ≥1 in 100 and <1 in 10 (≥1% and <10%)
Uncommon: ≥1 in 1,000 and <1 in 100 (≥0.1% and <1%)
Rare: ≥1 in 10,000 and <1 in 1,000 (≥0.01% and <0.1%)
*post-marketing

Adverse Events considered to be expected for Reporting Purposes in SAA

Blood and lymphatic system disorders Very common: Anemia
Gastrointestinal disorders Very common: Abdominal pain, diarrhea, nausea
General disorders and administrative conditions Very common: Dizziness, fatigue, febrile neutropenia, pyrexia
Hepatobiliary disorders Very common: Transaminases increased
Musculoskeletal and connective tissue disorders Very common: Arthralgia, muscle spasms, pain in extremity

Nervous systems disorders Very common: Headache
Respiratory, thoracic and mediastinal disorders Common: Cough, dyspnea, oropharyngeal pain, rhinorrhea
Skin and subcutaneous tissue disorders Very common: Ecchymosis
Vascular disorders Rare: post-marketing cases of Thrombotic microangiopathy with acute renal failure reported spontaneously

Adverse Events considered to be expected for Reporting Purposes in MDS/AML

Blood and lymphatic system disorders Very common: Leukocytosis**, white blood cell count increased
Gastrointestinal disorders Very common: Nausea, diarrhea, vomiting, constipation, abdominal pain
General disorders and administrative conditions Very common: Fatigue, pyrexia
Hepatobiliary disorders Uncommon: Drug-induced liver injury
Investigations Rare: Serum discoloration***
Nervous systems disorders Very common: Dizziness, Headache
Respiratory, thoracic and mediastinal disorders Very common: Cough
Skin and subcutaneous tissue disorders Common: Skin discoloration
Vascular disorders Very common: Hematoma

** Leukocytosis and white blood cell count increased occur individually with a frequency of common, however the terms were grouped as they represent the same medical concept, giving a revised frequency of very common.
*** Serum discoloration has been reported in investigator sponsored studies in MDS/AML, and can lead to analytical interference with some colorimetric analytical methods

12.3.4 Related to Corticosteroids

Corticosteroids can make the body retain water and salt, cause diabetes and acne, and worsen high blood pressure. In addition, they will probably increase your appetite and may cause insomnia or mood changes. Steroids can also cause stomach ulcers and soften bones, leading to osteoporosis. The more dangerous problems, like bone thinning, only occur with long-term use; the other side effects will stop when the medication is discontinued. A small number of cases of "aseptic

necrosis" have been observed when steroids have been used in high doses for durations comparable to those we will use in this study. Aseptic necrosis is a thinning of the bone near the joints, which can lead to chronic pain, sometimes requiring joint replacement. Steroids also suppress the immune system and raise your susceptibility to infection; taking CsA and steroids together increases this risk still further. We will ask that you plan to stay in the hospital for the first two weeks of your therapy so that infections, if they occur, can be treated promptly.

12.3.5 Related to bone marrow aspirate and biopsy

No major risks are involved with bone marrow aspirate and biopsy. However, a small risk of infections, pain, bleeding, and hematoma formation at the site of the aspiration exists with the procedure.

12.3.6 Related to general anesthesia (minors only)

The risks of general anesthesia include having a decreased breathing rate, aspiration, and low blood pressure.

12.3.7 Related to blood draws

No major risks are involved with blood draws. Minor complications including bleeding, pain, and hematoma formation at the site of blood draws, vasovagal reactions or infections may rarely occur.

12.3.8 Related to Cardiac Monitoring

An electrocardiogram (ECG) is a test that measures the electrical activity of the heartbeat. With each beat, an electrical impulse (or "wave") travels through the heart. This wave causes the muscle to squeeze and pump blood from the heart. A technician will put patches (electrodes) on the chest, arms and legs. The electrodes are soft and don't cause any discomfort when they're put on or taken off by the technician. The machine only records the ECG. It doesn't send electricity into the body. There's no pain or risk associated with having an electrocardiogram.

12.3.9 Related to Central Line Placement

A catheter may be placed in a large vein of the neck, chest, or arm using local anesthetic. Patients will sign a separate consent for the placement procedure. Only trained experienced staff will place the line in order to minimize these procedure related risks.

The risks from the procedure are low; they include bleeding, bruising, or infection at the site of insertion. Very rarely (less than 1% of the time), the line placement may nick a vein causing one lung to collapse during line insertion. If the lung collapses, a tube may have to be inserted into the chest and remain in place until the lung re-expands. Because of this risk, patients will have a chest x-ray following the procedure to make sure the line is in the correct place and that the lung is not collapsed. Once placed, the line will remain in place until drug administration is complete.

12.3.10 Related to Concomitant Medications

Pentamidine: cough (31-47%), bronchospasm (10-23%), decreased appetite (53-72%), fatigue, metallic taste, shortness of breath, decreased appetite, dizziness, rash, nausea, pharyngitis, chest pain/congestion, night sweats, chills, vomiting.

Valacyclovir: Nausea and/or vomiting, headache, dizziness, abdominal pain, dysmenorrhea, arthralgia, acute hypersensitivity reactions, elevations in liver enzyme laboratory values (e.g. AST). Renal failure and CNS symptoms have been reported in patients with renal impairment who

received valacyclovir at greater than the recommended dose.

12.3.11 Related to Genetic Testing

Privacy Risks Associated with Genetic Testing

Because this study involves collecting personal, identifiable information about you, there is a possibility that people who are not supposed to see this information might somehow gain access to it. We will take precautions to prevent this, but we cannot ever be certain that it won't happen. To minimize this chance, we will assign you a study number, use that number to label your samples and medical information, and save the information linking you to that study number in a secure file. All of the information we collect will be stored in a secure manner, such as in a locked cabinet or in password-protected computer files. Also, the type of genetic testing that we will be doing is not specific to your tissues and therefore could not be used to identify you or your family members.

12.4 RISKS IN RELATION TO BENEFIT

For adult subjects: The benefits to the subjects could be reduction or even abolition of transfusion requirements and/or improvement of cytopenia, resulting in improved quality of life and also decreased morbidity and mortality from transfusion-associated viral agents, iron overload, and/or a susceptibility to infections. Potentially, treatment with other more toxic therapies could also be avoided or postponed.

The benefits of this study and the acquisition of bone marrow and blood samples important for the understanding of the pathophysiology of immune-mediated bone marrow failure states have been described in the previous paragraphs.

For pediatric subjects:

(a) the risk is justified by the anticipated benefit to the subjects: We are offering pediatric subjects with a probably lethal hematological disease an alternative to symptomatic therapy.

(b) the relation of the anticipated benefit to the risk is at least as favorable to the subjects as that presented by available alternative approaches. The benefits to the patients could be reduction or even abolition of transfusion requirements and/or improvement of low peripheral blood counts, resulting in improved quality of life and also decreased morbidity and mortality from transfusion-associated viral agents, iron overload, and/or a susceptibility to infections. Potentially, treatment with other more toxic therapies could also be avoided or postponed.

(c) adequate provisions are made for soliciting permission of their parents or guardians, as set forth in 46.408.

For Cognitively Impaired:

This research provides the prospect of direct benefit; therefore, inclusion is justified. The benefits to the participants could be improvement of cytopenias resulting in improved quality of life and also decreased morbidity and mortality from transfusion-associated viral agents, iron overload, and/or a susceptibility to infections. Potentially, treatment with other more toxic therapies could also be avoided or postponed. Not allowing participants who cannot provide consent would deny them the potential benefits this protocol offers for their AA. There are no plans to include institutionalized participants.

12.5 INFORMED CONSENT PROCESSES AND PROCEDURES

Informed consent will be conducted following OHSRP Policy 301- Informed Consent.

An IRB-approved consent form will be provided to the participant electronically or by hard copy for review prior to consenting. The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved platforms). The investigational nature and objectives of this trial, the procedures, and their attendant risks and discomforts and potential benefits will be carefully explained to the participant in a private setting. The participant will be given as much time as they need to review the document and to consult with their family, friends, and personal health care providers. In addition, a study team member will be available to answer any questions.

A signed and dated informed consent document will be obtained by any investigator authorized to consent (See Key Study Personnel Page) prior to entry onto the study. Consent may be obtained with required signatures on the hard copy of the consent or on the electronic document.

When a document that is in electronic format is used for obtaining consent, this study may use the iMed platform which is 21 CFR, Part 11 compliant, to obtain the required signatures.

During the consent process, participants and investigators may view the same approved consent document simultaneously when participant is being consented in person at the Clinical Center or both may view individual copies of the approved consent document on screens in their respective locations remotely. Signatures may be obtained either by both directly signing on the device that the consenting investigator is using (when in person) or through iMed Mobile Signature Capture (remotely) which allows texting or emailing a link to the participant. That link allows the participant to review the consent, then proceed to sign on the device they are using.

Whether hard copy or electronic, both the investigator and the participant will sign the document with a hand signature using a pen (if using hard copy), finger, stylus, or mouse (if electronic).

When done remotely, if the participant prefers to sign a hard copy, they may be instructed to sign and date the consent document during the discussion and mail, secure email or fax the signed document to the consenting investigator.

Whether in person or remotely, the privacy of the participant will be maintained.

Finally, the fully signed informed consent document will be stored in the electronic medical record, and the participant will receive a copy of the signed informed consent document.

All patients will identify a local physician. NIH team will establish contact with the local physician to provide all protocol specific information and our contact information (Appendix A).

After the consent, study drug will be shipped to the patient or to the local treating physician within 24-48 hours. The patient will initiate medications as instructed upon receipt. Patient will notify us of the start date, which will be recorded in CRIS.

Pregnancy-related consent disclosures and documentation:
The consent form includes pregnancy-related risks and disclosures, which will be reviewed in a private setting at enrollment and, if applicable, when pregnancy is reported. Participants will have adequate time for questions; interpreter services will be offered as needed; understanding will be confirmed (e.g., teach-back); and the consent discussion, including pregnancy-related disclosures, will be documented in the medical record.

Minor participants: If the subject is a minor, the parent who signs the consent for the minor must be a legally recognized parent or guardian. For minors that are 7 years of age (inclusive) and up, where deemed appropriate by the clinician, and the child's parent or guardian, the child will also be included in all discussions about the trial. Typically, assent will be obtained as appropriate based on age and taking into consideration the child's level of understanding and maturity.

1. Children under the age of 7 years (exclusive): Will not be required to provide assent as they typically do not have the ability to fully understand the nature of research.
2. Children who are 7 years of age (inclusive) but less than 12 years of age (exclusive): Verbal assent will be obtained as appropriate.
3. Children who are 12 years of age (inclusive) but less than 18 years of age (exclusive): Will be asked to sign an assent form.

The parent or guardian will sign on the designated line on the informed consent providing permission for their child to participate. The consent/assent process will be documented in the child's medical record, including the assessment of the child's ability to provide assent (verbal versus written) as applicable.

In cases where parents share joint legal custody in making medical decisions of their child (e.g. by a custody agreement or court order) both parents must give their parental permissions regardless of level of risk of the research. Exceptions may be made if one parent is deceased, becomes incompetent or is not reasonably available (e.g. in prison).

We will inform potential adult and minor female participants during the consent and/or assent process that for safety, we need to do a pregnancy test. She will also be told that if it is positive, we will counsel her. If the patient does not want to proceed she will be advised not to sign the assent and her enrollment on this protocol will end.

If at any time during participation in the protocol, new information becomes available relating to risks, adverse events, or toxicities, this information will be provided orally or in writing to each enrolled or prospective patient. Documentation will be provided to the IRB and if necessary, the informed consent amended to reflect relevant information.

12.5.1 Consent for minors when they reach the age of majority:

When a pediatric subject reaches age 18, continued participation will require consenting of the now adult with the standard protocol consent document to ensure legally effective informed consent has been obtained. Should sample or data analysis continue following completion of active participation and the subject has reached 18 years of age, we will attempt to contact the subject using the last known contact information to obtain consent for continued use of data or samples

collected during their prior visit. Given the length of time that may have transpired for some of the subjects since their last visit for this study, we request waiver of informed consent for those individuals who after good faith efforts to contact them, we are unable to contact.

Requirements for Waiver of Consent consistent with 45 CFR 46.116 (d), each of which must be addressed in relation to the protocol:

(1) The research involves no more than minimal risk to the subjects;

a) Analysis of samples and data from this study involves no additional risks to subjects.

(2) The waiver or alteration will not adversely affect the rights and welfare of the subjects;

a) Samples and data will be kept in secure locations in the laboratory of Dr. Young. Retention of samples or data does not affect the welfare of subjects.

(3) The research could not practicably be carried out without the waiver or alteration; and

a) Considering the length of time between a minor's enrollment and their age of majority, it is possible that more than a few subjects may be lost to follow up. A significant reduction in the number of samples analyzed could impact the quality of the research.

(4) Whenever appropriate, the subjects will be provided with additional pertinent information after participation.

a) We only plan to request a waiver of reconsent for those subjects who have been lost to follow-up.

12.5.2 Informed consent for adult research participants unable to provide consent:

If there is an unexpected enrollment of a research participant unable to provide informed consent, the procedures per NIH HRPP Policy 403 will be used to enroll these participants in this protocol.

Consent and Assent:

Procedures to determine capacity: If documentation of decision making capacity is not present in the medical record or the investigator questions the decision-making capacity of the individual, then the Ability to Consent Assessment Team (ACAT) (301-496-9675 or 301-496-2429) will be contacted to make the determination.

Procedures for obtaining consent from legally authorized representative (LAR) will be in accordance with NIH HRPP Policy 403.

12.6 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s). This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the NHLBI. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by NHLBI research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the NHLBI.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

12.7 HUMAN DATA SHARING, INCLUDING GENOMIC DATA SHARING, AND PUBLICATION

12.7.1 NIH Data Management and Sharing Policy and NIH Genomic Data Sharing Policy Compliance

This study will comply with the NIH Data Management and Sharing (DMS) Policy, which applies to all new and ongoing NIH-funded research in the IRP, as of January 25, 2023, that is associated with a ZIA, with a clinical protocol that undergoes scientific review and/or will involve genomic data sharing.

The Genomic Data Sharing (GDS) Policy does not apply to this study. However, genetic data collected under this protocol may be deposited in an open or controlled access database and shared, as required by scientific journals.

12.7.2 NIH Public Access Policy Compliance

This study will comply with the NIH Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

12.8 CONFLICT OF INTEREST

The Principal Investigator assured that each associate investigator listed on the protocol title page received a copy of the NIH's Guide to preventing conflict of interest. Investigators added subsequent to the initial circulation were provided a copy of the document when they were added. Copies of the Conflict of Interest Statement were forwarded to the Clinical Director. No initial or subsequent members of the research team reported a potential conflict of interest.

12.9 COLLABORATION(S)

Danielle Townsley, M.D. will collaborate on trial with protocol related questions and interpretation of study data. Dr. Townsley has no access to PII and is not participating in direct patient care.

Phillip Scheinberg, M.D. will collaborate on trial with protocol related questions and interpretation of study data. Dr. Scheinberg has no access to PII and is not participating in direct patient care.

12.10 TECHNICAL TRANSFER AGREEMENTS

This protocol has a CRADA.

13 PHARMACEUTICALS

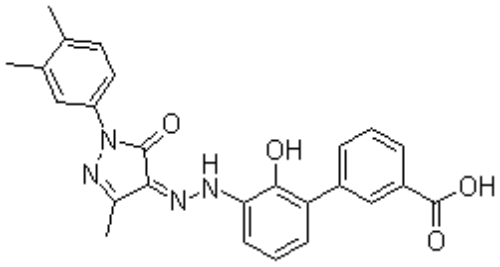
13.1 ELTROMBOPAG (PROMACTA®)

Chemical Name: The chemical name for eltrombopag olamine is 3'-{(2Z)-2-[1-(3,4-dimethylphenyl)-3-methyl-5-oxo-1,5-dihydro-4H-pyrazol-4-ylidene]hydrazino}-2'- hydroxy-3-biphenylcarboxylic acid - 2-aminoethanol (1:2).

Molecular formula: C25H22N4O4.2(C2H7NO).

Molecular weight is 564.65 for eltrombopag olamine and 442.5 for eltrombopag free acid.

Chemical and structural formula:



Physical form: red/brown solid.

Solubility: Eltrombopag olamine is practically insoluble in aqueous buffer across a pH range of 1 to 7.4, and is sparingly soluble in water.

Supply: The drug Novartis is providing for this study will be commercial material, based on their supply. It is available as tablets of 12.5, 25, 50 and 75mg. It is also in the process of being manufactured as a powder for oral suspension.

- **Tablets:**

  White, round, film-coated tablets without debossing are provided, containing eltrombopag olamine equivalent to 12.5 mg, 25 mg, 50 mg, or 75 mg of eltrombopag free acid. Placebos to match the active tablets are available. Tablets are packaged in white HDPE bottles with white plastic, induction-seal, child-resistant caps.

  Green, oval, film-coated tablets debossed with 'SLC' on one side are provided, containing eltrombopag olamine equivalent to 200 mg or 300 mg of eltrombopag free acid. A placebo to match the active tablets is available. Tablets are packaged in white HDPE bottles with white plastic, induction-seal, child-resistant caps. Desiccant may be included.

  Commercial image actives (12.5 mg - white, 25 mg- orange or white, 50 mg blue or brown, 75 mg – pink) which are equivalent to the clinical forms with the exception of the film coated color may also be provided for clinical use. These forms are also packed in white HDPE bottles with white plastic, induction -seal, child-resistant caps. Desiccant may be included. Additionally, the commercial image tablets may be provided in aluminum foil blister packages.

- **Powder for Oral Suspension:**

  Doses for children between the ages 4 to 5 (2.5mg/kg non-East/South Asian participants, and 1.25mg/kg East/South Asian participants) will be provided in the form of sachets (when available). The powder for oral suspension is a reddish brown to yellow powder in a sachet. Two PfOS strengths are available, containing eltrombopag olamine equivalent to 12.5 mg and 25 mg of eltrombopag free acid. Reconstitution of eltrombopag PfOS will follow instructions outlined in the Investigator's Brochure and Package Insert.

Stability: Store, both tablets and powder, at 25°C (77°F); excursions permitted to 15o to 30°C (59o to 86°F) [*see USP Controlled Room Temperature*].

Shipping: The NIH Investigational Drug Management and Research Section (IMDRS) will be responsible for receiving, storing, dispensing and accounting for drug product. The shipping address for Novartis supplied investigational agent is:

National Institutes of Health

IMDRS, Room 1C230
10 Center Drive, MSC 1196, Building 10 Bethesda, Maryland 20892-1196

Accountability Procedures: Drug accountability records will be maintained for all clinical supplies. All empty and partially used vials and clinical trial supplies will be destroyed locally according to the institution's standard operating procedures for drug destruction. The pharmacy will maintain detailed documentation of the number and identification of vials, which are destroyed, and copies of these documents will be provided to the Sponsor and Novartis. Disposition of all unused boxes of study drug will be carried out according to instructions provided by the Sponsor and/or Novartis at the end of the study after drug accountability is performed.

13.2 CYCLOSPORINE (GENGRAF, SANDIMMUNE, NEORAL)

Supply: Cyclosporine will be obtained by the NIH Clinical Center Pharmacy Department from commercial sources and is available in capsules (25 mg and 100 mg), USP [MODIFIED], oral solution (100 mg/ml), USP [MODIFIED], and as a parenteral concentrate for injection (50 mg/ml). When oral capsules are prescribed for this protocol, the cyclosporine capsules, USP [NON-MODIFIED] should NOT be used.

Preparation: For parenteral doses, each milliliter of concentrate (50mg/ml) should be diluted in 20 to 100ml of dextrose 5% in water or sodium chloride 0.9%. Parenteral doses of cyclosporine will be prepared in non-PVC containers and infused with non-PVC administration sets/tubing. The recommended liquids for dilution of the oral solution to improve palatability include milk, chocolate milk or orange juice, preferable at room temperature.

Storage and Stability: Capsules, oral solution, and ampules of parenteral concentrate bear expiration dates and are stored at room temperature and protected from light. Cyclosporine concentrate for injection that has been diluted to a final concentration of approximately 2mg/ml is stable for 24 hours in 5% dextrose or 0.9% sodium chloride injection in glass, PVC or non-PVC plastic containers. To minimize the potential for sorption to PVC plastic bags and tubing as well the leaching of phthalate plasticizer (DEHP) into the solution, only non-PVC plastic bags and intravenous administration sets should be utilized.

Administration: Cyclosporine may be given intravenously or orally.

Toxicities: see section 12.4.2 Risks related to CsA

13.3 ANTI-THYMOCYTE GLOBULIN (EQUINE) STERILE SOLUTION (ATGAM®)

Other: Antithymocyte Gammaglobulin, Antithymocyte Globulin, ATGAM, Antithymocyte Immunoglobulin, lymphocyte immune globulin and h-ATG

Supply / availability: commercially available (Pharmacia & Upjohn Company)

Product description: Anti-thymocyte globulin (equine) sterile solution (ATGAM®) is available in 5 ml ampoules containing 50 mg of horse gamma globulin/mL (250 mg per ampoule).

Preparation: The calculated dose of anti-thymocyte globulin should be diluted in 0.9% sodium chloride injection to a concentration not to exceed 4 mg/mL.

Storage / stability: Anti-thymocyte globulin (equine) ampoules should be stored in a refrigerator at 2^{o} to 8^{o} C. Once diluted, anti-thymocyte globulin (equine) is physically and chemically stable for up to 24 hours at concentrations of up to 4 mg/mL in the recommended diluents. It is

recommended that diluted anti- thymocyte globulin (equine) be stored in a refrigerator if it is prepared prior to the time of infusion.

Administration: Anti-thymocyte globulin (equine) should be administered into a high-flow central vein through an in-line filter with a pore size of 0.2 to 1 micron. The dose should be infused over no less than 4 hours. Infusion times may be extended to up to 24 hours for intolerance. Patients should be closely monitored for infusion / allergic reactions.

Compound: Principally monomeric IgG, prepared from plasma or serum of healthy horses hyperimmunized with human thymus lymphocytes.

Action: Immunosuppressive agent. Exact mechanism of immunosuppression of ATGAM has not been fully elucidated but may involve elimination of antigen-reactive T-cells in peripheral blood and/or alteration of T-cell function.

Side effects: see section 12.4.1 Risks related to h-ATG

13.4 PENTAMIDINE

Supply: Commercially available (NebuPent®, American Pharmaceutical Partners, Inc.).

Product description: Pentamidineisethionate is available as a 300 mg single dose vial containing 300 mg of lyophilized powder in a 15 mL capacity vial. The contents of one vial must be dissolved in 6 mL of sterile water for injection, USP. It is important to use only sterile water; saline solution will cause the drug to precipitate.

Storage and stability: Store dry product at controlled room temperature 15-30^{O}C (59-86^{O}F).

Route of administration: Inhalation; Once reconstituted, the entire contents of a vial should be placed into the Respigard® II nebulizer (Marquest) reservoir for administration by inhalation. Do not mix the pentamidine solution with any other drugs.

Toxicities: see section 12.4.10 Risks related to Concomitant medications.

13.5 VALACYCLOVIR

Generic name: valacyclovir

Brand Name: Valtrex

Supply: Commercially available.

Pharmacology: Valacyclovir is the hydrochloride salt of L-valyl ester of the antiviral drug acyclovir. After oral administration, valacyclovir is rapidly absorbed from the GI tract and nearly completely converted to acyclovir and L-valine by first-pass intestinal or hepatic metabolism.

Product description: Valacyclovir is available in 500mg tablets and 1gm tablets. Dose adjustment is necessary in patients with significant renal impairment (refer to the manufacturer's labeling for dose adjustment guidelines.

Storage and Stability: Oral tablets should be stored at 15^{O} to 25^{O}C (59^{O} to 77^{O}F). *Route of administration:* Oral

Toxicities: see section 12.4.10 risks related to concomitant medications.

13.6 FDA REGULATORY CONSIDERATION

An IND application with the FDA is not required for this study. The proposed research with Eltrombopag meets the exemption requirements noted in 21 CRF 312.2, specifically:

1. The drug product is lawfully marketed in the United States.
2. The investigation is not intended to be reported to FDA as a well-controlled study in support of a new indication and there is no intent to use it to support any other significant change in the labeling of the drug.
3. In the case of a prescription drug, the investigation is not intended to support a significant change in the advertising for the drug.
4. The investigation does not involve a route of administration, dose, patient population, or other factor that significantly increases the risk (or decreases the acceptability of the risk) associated with the use of the drug product (21 CFR 312.2(b)(1)(iii)).
5. The investigation is conducted in compliance with the requirements for review by an IRB (21 CFR part 56) and with the requirements for informed consent (21 CFR part 50).
6. The investigation is conducted in compliance with the requirements of § 312.7 (i.e., the investigation is not intended to promote or commercialize the drug product).

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APPENDIX A: GUIDE FOR LOCAL PHYSICIAN

1. CYCLOSPORINE (CsA)
    a. Dose: 2mg/kg daily by mouth
    b. Administration: To be taken consistently in relation to time of day and meals. *Recommend taking it in the morning same time every day with food
    c. Supportive medication considerations:
        i. Magnesium: Patients are expected to have magnesium wasting. Weekly labs should include magnesium levels and low levels need to be treated with magnesium supplementation (Goal >2mg/mL)
    d. Food/Medication interactions
        i. Various agents are known interfere with plasma cyclosporine concentrations usually by inhibition or induction of CYP3A4
        ii. Common drugs: Erythromycin, ketoconazole, diltiazem, rifampin, phenytoin, and phenobarbital. Refer to FDA label when prescribing new medications if unsure regarding possible interactions. Avoid using concomitantly with cyclosporine.
        iii. Avoid Grapefruit juice and grapefruit
    e. Clinical and Laboratory monitoring
        i. Common side effects: Headaches, hypertension, gastrointestinal upset, nausea. Posterior reversible encephalopathy syndrome (PRES) is a rare side effect.

            *Recommend Amlodipine for hypertension (Goal <120/80mm/Hg)

            *Recommend ondansetron and pantoprazole for nausea/GI upset
        ii. Renal panel including magnesium at least weekly
        iii. Cyclosporine level per investigators at NIH or local physician as clinically indicated only
    f. Dose Delays, Modifications or Discontinuation
        i. Discuss with investigators prior to dose alteration or discontinuation unless medically emergent. Please communicate any changes immediately.
        ii. Common reasons: renal impairment, severe gum hyperplasia, severe headaches, hyperkalemia

2. ELTROMBOPAG
    a. Dose:

Dosing according to age and ethnicity

Age groups (years)	Daily dose
Non-Asian	
12-85	150 mg
6-11	75 mg
2-5	2.5 mg/kg
East Asian, South East Asian	
12-85	75 mg
6-11	37.5 mg
2-5	1.25 mg/kg

b. Administration

   i. Take on empty stomach (1 hour before or 2 hours after a meal) or with food containing no calcium or dairy products

   ii. Allow at least 4-hour interval between eltrombopag and other medications containing polyvalent cations (e.g. calcium, magnesium, aluminum, zinc, selenium or iron)

*** RECOMMEND – EARLY MORNING (4-5AM) IF BREAKFAST PLANNED AFTER 7AM AND DINNER COMPLETED BEFORE MIDNIGHT**

c. Food/ Medication interactions

   i. Strong inhibitors of cytochrome p450 may enhance eltrombopag activity – recommend to not use it

   ii. Examples: Trimethoprim, ciprofloxacin, and gemfibrozil

   iii. Food: Dairy, calcium and other polyvalent cations (as listed above)

d. Clinical and laboratory monitoring

   i. Skin rash – typically severe diffuse drug rash

   ii. Complete blood counts and hepatic panel at least twice weekly x 2 weeks than weekly if clinically acceptable

e. Dose Delays, Modifications or Discontinuation (discuss with investigators immediately prior to making any changes)

i. If ALT > 6 times ULN, repeat in 3 days Hold eltrombopag if ALT still >6 times ULN. Once ALT < 5 times ULN, restart eltrombopag at a dose level that is 25mg/day lower than the prior dose

ii. Discontinue eltrombopag if deep venous thrombosis (other than a line related upper extremity thrombosis), pulmonary embolism, TIA/stroke, or myocardial infarction

iii. Discontinue eltrombopag if diffuse skin rash

iv. Decrease dose as follows for over correction of platelets

Platelet Count	**Dose Adjustment or Response**
>200,000/μL (untransfused) at any time on study	Decrease dosage by 25mg every 2 weeks to lowest dosage that maintains platelet count ≥ 50,000/μL. In children under 12, the dose will be decreased by 12.5 mg.
>400,000/μL (untransfused) at any time on study	Discontinue eltrombopag for one week, if platelets fall to <200,000/μL; restart at dosage decreased by 25 mg/day (or 12.5 mg in children under 12).

3. Acceptable supportive treatment while on study
    a. Blood and platelet transfusions
        i. Platelet transfusions are recommended when:
            1. PLT level is <10 × 109/L, or
            2. PLTs <20 × 109/L and with active bleeding, or
            3. Per local standard of care (SOC), or as medically necessary.
        ii. RBC transfusions are recommended when:
            1. Hgb <7 g/dL, or if symptomatic according to local SOC.
            2. Infection management
    b. Hematopoietic growth factors (e.g., G-CSF, GM-CSF, or erythropoietin) if deemed necessary. DO NOT ADMINISTER Romiplostim (N-Plate) or IL-11 (Neumega)
    c. Oral contraceptives or Leuprolide acetate for menses suppression
    d. Antibiotics, anti-fungals and anti-virals are permitted as clinically indicated. The local medical guidelines for treatment of infections/febrile neutropenia should be followed.
4. Laboratory monitoring for cytopenias

a. CBC weekly and reticulocyte (if testing available) weekly

b. Transfusion goals: Hb >8g/dL or otherwise clinically indicated, plt >10μL or otherwise clinically indicated

5. Neutropenic fever management

a. Single temperature of 38.5°C or two readings of 38.0°C or greater will need hospital admission to begin prompt broad-spectrum empiric antibiotics per current infectious disease guidelines. Full infectious work up required to identify source.

b. Notify the NIH care team immediately

Contacts:

Please contact us if you have any questions or concerns about any of the above information.

The main point of contact is study coordinator Olga Rios, RN. She can be contacted at 301-496-4462 or emailed at olga.rios@nih.gov.

Other points of contact include:

Principal Investigator: Bhavisha Patel, MD: Ph 301-402-3477, Email Bhavisha.patel@nih.gov

Associate Investigator: Emma Groarke, MD: Ph 301-451-7129, Email Emma.Groarke@nih.gov

Associate Investigator: Neal Young, MD: Ph 301-496-5093, Email youngns@nhlbi.nih.gov

APPENDIX B: TELEPHONE WEEKLY FOLLOW UP

1. Clinical assessment
    a. Disease related: fever, localizing infectious symptoms, bleeding, fatigue
    b. Cyclosporine related: headache, nausea/other gastrointestinal symptoms, gum hyperplasia, high blood pressure
    c. Eltrombopag related: skin rash, swelling/pain of any extremities, chest pain, shortness of breath, yellowing of the skin or eyes
    d. Compliance to medication
    e. Evaluation by any physicians, emergency department, or admission
    f. Address other complains by patients

2. Laboratory assessment
    a. Complete blood count
    b. Chemistry panel
    c. Liver function test
    d. Transfusion requirement

3. Medications
    a. Review medication list and update as needed in CRIS

4. Local physician involvement
    a. Will discuss any concerning findings on telephone interview with the local physician and discuss work up and/or inform changes to the treatment regimen that were recommended to the patient
    b. Brief note will be placed in CRIS if any changes to the treatment plan made